

# Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/US05/005221

International filing date: 18 February 2005 (18.02.2005)

Document type: Certified copy of priority document

Document details: Country/Office: US  
Number: 60/546,385  
Filing date: 20 February 2004 (20.02.2004)

Date of receipt at the International Bureau: 17 March 2005 (17.03.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)



World Intellectual Property Organization (WIPO) - Geneva, Switzerland  
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse

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**APPLICATION NUMBER: 60/546,385**

**FILING DATE: February 20, 2004**

**RELATED PCT APPLICATION NUMBER: PCT/US05/05221**



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18379 U.S. PTO

PTO/SB/16 (01-04)

Approved for use through 07/31/2006. OMB 0651-0032

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Express Mail Label No. ER 828466182 US

22859 U.S. PTO  
60/546385

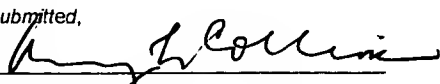
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TITLE OF THE INVENTION (500 characters max)					
Novel Apo2 and IL-24 Polypeptides, Polynucleotides and Methods of Their Use					
Direct all correspondence to: CORRESPONDENCE ADDRESS					
<input type="checkbox"/> Customer Number: <div style="border: 1px solid black; width: 200px; height: 20px;"></div>					
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ENCLOSED APPLICATION PARTS (check all that apply)					
<input checked="" type="checkbox"/> Specification Number of Pages		93		<input type="checkbox"/> CD(s), Number	
<input checked="" type="checkbox"/> Drawing(s) Number of Sheets		4		<input checked="" type="checkbox"/> Other (specify) Abstract	
<input type="checkbox"/> Application Data Sheet. See 37 CFR 1.76					
METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT					
<input checked="" type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27.				FILING FEE Amount (\$)	
<input checked="" type="checkbox"/> A check or money order is enclosed to cover the filing fees.				<div style="border: 1px solid black; padding: 10px; text-align: center;">\$80.00</div>	
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[Page 1 of 2]

Respectfully submitted,

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2/20/2004

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(if appropriate)

Docket Number: FP20040007

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Docket Number **FP20040007**

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Number 2 of 2

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UNITED STATES PROVISIONAL PATENT APPLICATION

for

NOVEL APO2 AND IL-24 POLYPEPTIDES, POLYNUCLEOTIDES AND  
METHODS OF THEIR USE

by

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**NOVEL APO2 AND IL-24 POLYPEPTIDES, POLYNUCLEOTIDES AND  
METHODS OF THEIR USE**

**FIELD OF THE INVENTION**

[0001] The present invention relates to newly identified Interleukin 24 (“IL-24”) and Apo2 splice variant molecules, their polypeptide sequences, the polynucleotides encoding the polypeptide sequences, vectors, host cells, compositions and kits containing such, modulators thereof, their use in the treatment of diseases, and utility in identifying agonists, antagonists and receptors thereto, as well as the production thereof.

**BACKGROUND OF THE INVENTION**

[0002] Control of cell numbers in mammals is believed to be determined, in part, by a balance between cell proliferation and cell death. One form of cell death, sometimes referred to as necrotic cell death, is typically characterized as a pathologic form of cell death resulting from some trauma or cellular injury. In contrast, there is another, “physiologic” form of cell death that usually proceeds in an orderly or controlled manner. This orderly or controlled form of cell death is often referred to as “apoptosis” (Barr et al., *Bio/Technology*, 12:487-493 (1994); Steller et al., *Science*, 267:1445-1449 (1995)).

[0003] Apoptotic cell death naturally occurs in many physiological processes, including embryonic development and clonal selection in the immune system (Itoh et al., *Cell*, 66:233-243 (1991)). Decreased levels of apoptotic cell death have been associated with a variety of pathological conditions, including cancer, lupus and herpes virus infection (Thompson, *Science*, 267:1456-1462 (1995)). Increased levels of apoptotic cell death may be associated with a variety of other pathological conditions, including AIDS, Alzheimer’s disease, Parkinson’s disease, amyotrophic lateral sclerosis, multiple sclerosis, retinitis pigmentosa, cerebellar degeneration, aplastic anemia, myocardial infarction, stroke, reperfusion injury, and toxin-induced liver disease (Thompson, 1995).

[0004] IL-24, also referred to as melanoma differentiation-associated gene 7 (“Mda-7”), is a cytokine related to the IL-10 family. IL-24 was first identified due to its elevated expression in growth arrested and terminally differentiated human melanoma cells

(Ekmekcioglu et al., *Int. J. Cancer*, 94(1):54-59 (2002); Madireddi et al., *Adv. Exp. Med. Biol.*, 465:239-261 (2000)). Apo2 ligand, also known as TRAIL, is a member of the tumor necrosis factor ("TNF") cytokine family (Marsters et al., *Recent Prog. Horm. Res.*, 54:225-234 (1999)).

[0005] Both Apo2 and IL-24 have been found to be associated with diseases. However, the cause or effect of their presence in disease is unclear. It would be highly desirable to clarify the function and utility of Apo2 and IL-24 molecules, as they may provide an additional means of controlling diseases, as well as providing further insight into the development of self-tolerance by the immune system and the etiology of cancers, immunity diseases, infectious diseases, and ischemia related disorders.

#### SUMMARY OF THE INVENTION

[0006] The present invention provides newly identified Apo2 and IL-24 variant polypeptides, as well as isolated polynucleotides encoding the polypeptides and expression vectors containing the isolated polynucleotides. Properties of the disclosed Apo2 and IL-24 variants include the ability to induce apoptosis of certain types of target cells. Among the types of cells that are killed by contact with the molecules of the invention are, for example, cancer cells such as leukemia, lymphoma, and melanoma or other tumor cells, and cells infected with a virus.

[0007] It is, thus, an object of the present invention to provide methods and compositions for treatment, prevention and diagnosis of diseases or conditions associated with the polypeptides of the invention, as well as the polynucleotides encoding the polypeptides.

[0008] In another object of the invention, a method for producing the disclosed polypeptides is provided and involves, for example, cell free expression and culturing host cells transformed with a recombinant expression vector that contains the polypeptides-encoding nucleic acids under conditions appropriate for expression of polypeptides, then recovering the expressed polypeptides from the culture.

[0009] It is another object of the present invention to provide modulators of the polypeptides of the invention, including but not limited to antibodies thereto, for treatment, prevention and diagnosis of diseases or conditions associated with their respective receptors.

- [0010] It is yet another object of the present invention to identify further uses for the polypeptides of the invention, as well as the isolated polynucleotides encoding the polypeptides and modulators thereto.
- [0011] Thus, in accordance to one of the objects of the invention, there is provided compositions containing the polypeptides of the invention, and a vehicle such as pharmaceutically acceptable carrier or excipient, wherein the compositions are useful for treatment or prophylaxis of diseases in animals.
- [0012] In accordance to a further one of the objects of the present invention, there is provided modulators as above, wherein the modulators are antibodies.
- [0013] In accordance to yet another one of the objects of the present invention, there is provided antibodies that specifically bind to or interfere with the activity of polypeptides of the invention, wherein such polypeptides contain at least a sequence of 6 contiguous amino acid residues chosen from among SEQ ID NOs. 6-10 and 15.
- [0014] In accordance to another one of the objects of the present invention, there is provided a method for stimulation of an immune response in a subject by use of the polypeptides of the invention, as well as the isolated polynucleotides encoding the polypeptides.
- [0015] In accordance to another one of the objects of the present invention, there is provided a method for treating or preventing an infection in a subject by use of the polypeptides of the invention, as well as the isolated polynucleotides encoding the polypeptides.
- [0016] In accordance to a further one of the objects of the present invention, there is provided a method for modulating an immune response in a subject by use of an agonist or an antagonist of the polypeptides of the invention.
- [0017] In accordance to another one of the objects of the present invention, there is provided a method of treatment of diseases, such as inflammatory diseases, autoimmune diseases, ischemia related disorders, such as stroke, myocardial infarction, and fulminant liver failure, cancer, and infectious diseases.
- [0018] The invention identifies nucleotide and polypeptide targets for diagnosis and therapeutic intervention of the disease states described herein, and provides methods for



diagnosis and treating these diseases by intervening with these targets. The invention provides the nucleic acid and amino acid sequences of these targets in Appendices A-F.

[0019] In accordance with one of the objectives of the present invention, there is provided:

[0020] 1. A first isolated nucleic acid molecule comprising a first polynucleotide sequence chosen from among: (A) SEQ ID NOs. 1-5, 11-14, and 16; (B) a polynucleotide sequence encoding a polypeptide of SEQ ID NOs. 6-10 and 15; and (C) biologically active fragments thereof, as set forth in Appendices A-F.

[0021] 2. The nucleic acid molecule of 1, wherein the nucleic acid molecule is chosen from among: a cDNA molecule, genomic DNA molecule, a cRNA molecule, a siRNA molecule, a RNAi molecule, and an mRNA molecule.

[0022] 3. A double-stranded isolated nucleic acid molecule comprising the first nucleic acid molecule of 1 and its complement.

[0023] 4. A second isolated nucleic acid molecule comprising a second polynucleotide sequence that is at least 95% homologous to the first nucleic acid molecule of 1.

[0024] 5. A second isolated nucleic acid molecule comprising a second polynucleotide sequence that hybridizes to the first polynucleotide sequence of 1 under high stringency conditions.

[0025] 6. The second isolated nucleic acid molecule of 5, wherein the second polynucleotide sequence is complementary to the first polynucleotide sequence.

[0026] 7. The second isolated nucleic acid molecule of 6 wherein the second polynucleotide sequence is chosen from among an RNAi, an anti-sense molecule, and a ribozyme.

[0027] 8. An isolated polypeptide comprising an amino acid sequence, wherein the amino acid sequence is chosen from among SEQ ID NOs. 6-10 and 15 and active fragments thereof.

[0028] 9. An isolated polypeptide encoded by the first nucleic acid molecule of 1.

[0029] 10. A vector comprising the nucleic acid molecule of 1 or 9 and a promoter that regulates the expression of the nucleic acid molecule.

[0030] 11. The vector of 10, wherein the promoter is selected from one that is naturally contiguous to the nucleic acid molecule and one that is not naturally contiguous to the nucleic acid molecule.

- [0031] 12. The vector of 10, wherein the promoter is selected from an inducible promoter, a conditionally-active promoter (such as the cre-lox promoter), a constitutive promoter, and a tissue-specific promoter.
- [0032] 13. A recombinant host cell comprising the isolated nucleic acid of 1, the isolated polypeptide of 7, or the vector of 10.
- [0033] 14. The host cell of 13, wherein the cell is selected from a prokaryotic cell and a eukaryotic cell.
- [0034] 15. The host cell of 14, wherein the cell is a eukaryotic cell, and the eukaryotic cell is selected from the group consisting of a human cell, a non-human mammalian cell, an insect cell, a fish cell, a plant cell, and a fungal cell.
- [0035] 16. A non-human animal injected with the nucleic acid molecule of 1.
- [0036] 17. A non-human animal transformed with the nucleic acid molecule of 1.
- [0037] 18. A non-human animal injected with the polypeptide of 7.
- [0038] 19. A nucleic acid composition comprising the nucleic acid molecule of 1 and a carrier.
- [0039] 20. The nucleic acid composition of 19, wherein the carrier is pharmaceutically acceptable carrier or a buffer.
- [0040] 21. A polypeptide composition comprising the polypeptide molecule of 7 and a carrier.
- [0041] 22. The polypeptide composition of 21, wherein the carrier is pharmaceutically acceptable carrier or an excipient.
- [0042] 23. A vector composition comprising the vector of 10 and a carrier.
- [0043] 24. The vector composition of 23, wherein the carrier is pharmaceutically acceptable carrier or a buffer.
- [0044] 25. A host cell composition comprising the host cell of 13 and a carrier.
- [0045] 26. The host cell composition of 25, wherein the carrier is pharmaceutically acceptable or a buffer.
- [0046] 27. A method of producing a recombinant host cell comprising the steps of:
- (a) providing a composition comprising a vector that comprises the nucleic acid molecule of 1;
  - (b) allowing a host cell to come into contact with the vector to form

a recombinant host cell.

- [0047] 28. A method of producing a polypeptide comprising the steps of:
- (a) providing a composition comprising the recombinant host cell of 13;
  - (b) culturing the recombinant host cell to produce the polypeptide.
- [0048] 29. A method of producing a polypeptide comprising the steps of:
- (a) providing the nucleic acid of 1; and
  - (b) expressing the nucleic acid molecule in a cell free expression system to produce the polypeptide.
- [0049] 30. The method of 29, wherein the cell free expression system is selected from the group consisting of a wheat germ lysate expression system, a rabbit reticulocyte expression system, and a E. coli lysate expression system.
- [0050] 31. A diagnostic kit comprising a composition comprising a polynucleotide molecule that is complementary to the nucleic acid molecule of 1 and a vehicle.
- [0051] 32. A diagnostic kit comprising an antibody that specifically binds to the polypeptide of 7 or a biologically active fragment thereof.
- [0052] 33. A diagnostic kit comprising the polypeptide of 7 or biologically active fragments thereof.
- [0053] 34. A method of determining presence of the nucleic acid molecule of 1 or its complement comprising the steps of:
- (a) providing a complement to the nucleic acid molecule of 1 or the nucleic acid molecule of 1;
  - (b) allowing the molecules to interact; and
  - (c) determining whether interaction has occurred.
- [0054] 35. A method of determining the presence of an antibody specific to the polypeptide of 7 in a sample, comprising the steps of:
- (a) providing a composition comprising the polypeptide;
  - (b) allowing the polypeptide to interact with the sample; and
  - (c) determining whether interaction has occurred between the polypeptide and the antibody.
- [0055] 36. An antibody that specifically binds to or interferes with activity of the polypeptide of 7.

- [0056] 37. The antibody of 36, wherein the antibody is a selected from the group consisting of: polyclonal antibodies; monoclonal antibodies; single chain antibodies; and active fragments thereof.
- [0057] 38. The antibody of 36, wherein the antibody is a fragment and the fragment is selected from the group consisting of: an antigen binding fragment an Fc fragment, a cdr fragment, and a framework fragment.
- [0058] 39. A method of inhibiting tumor growth comprising the steps of:
- [0059] (a) providing a composition comprising the polypeptide chosen from among any of 7-8, 21, 28-29, or an active fragment thereof; and
- [0060] (b) contacting the tumor with the composition.
- [0061] 40. A method for killing tumor cells, the method comprising:  
contacting tumor cells having a death domain receptor with a polypeptide chosen from among any of 7-8, 21, 28-29 or an active fragment thereof.
- [0062] 41. The method of 40, wherein the tumor cells are human tumor cells.
- [0063] 42. The method of 41, wherein the tumor cells are solid tumor cells or a leukemia.
- [0064] 43. The method of 42, wherein tumor cells selected from the group consisting of: a carcinoma, a mammary adenocarcinoma, and a non-small cell lung carcinoma.
- [0065] 44. The method of 42, wherein the tumor cells are selected from the group consisting of: a breast tumor, a colon tumor, a lung tumor, a prostate tumor, a bladder tumor, a stomach tumor, and skin cancer.
- [0066] 45. A method for killing tumor cells, the method comprising:  
contacting the tumor cells with the polypeptide chosen from among any of 7-8, 21, 28-29 or an active fragment thereof.
- [0067] 46. The method of 45, wherein the tumor cells are solid tumor cells or a leukemia.
- [0068] 47. The method of 46, wherein tumor cells selected from the group consisting of: a carcinoma, a mammary adenocarcinoma, and a non-small cell lung carcinoma.
- [0069] 48. The method of 46, wherein the tumor cells are selected from the group consisting of: a breast tumor, a colon tumor, a lung tumor, a prostate tumor, a bladder tumor, a stomach tumor, and skin cancer.
- [0070] 49. A method for treatment of a mammary adenocarcinoma in a subject comprising the steps of:

[0071] (a) providing a composition containing the polypeptide chosen from among any of 7-8, 21, 28-29 or an active fragment thereof, and a pharmaceutically acceptable carrier; and

[0072] (b) administering the composition to the subject.

[0073] 50. A method for treatment of a non-small cell lung carcinoma in a subject comprising the steps of:

(a) providing a composition containing the polypeptide chosen from among any of 7-8, 21, 28-29 or an active fragment thereof, and a pharmaceutically acceptable carrier; and

(b) administering the composition to the subject.

[0074] 51. A method for treatment of a breast tumor in a subject comprising the steps of:

(a) providing a composition containing the polypeptide chosen from among any of 7-8, 21, 28-29 or an active fragment thereof, and a pharmaceutically acceptable carrier; and

(b) administering the composition to the subject.

[0075] 52. A method of treatment of a lung tumor in a subject comprising the steps of:

(a) providing a composition containing the polypeptide chosen from among any of 7-8, 21, 28-29 or an active fragment thereof, and a pharmaceutically acceptable carrier; and

(b) administering the composition to the subject.

[0076] 53. A method of treatment of a prostate tumor in a subject comprising the steps of:

(a) providing a composition containing the polypeptide chosen from among any of 7-8, 21, 28-29 or an active fragment thereof, and a pharmaceutically acceptable carrier; and

(b) administering the composition to the subject.

[0077] 54. A method of treatment of a tumor of the colon in a subject comprising the steps of:

(a) providing a composition containing the polypeptide chosen from among any of 7-8, 21, 28-29 or an active fragment thereof, and a pharmaceutically acceptable carrier; and

(b) administering the composition to the subject.

[0078] 55. A method of treatment of a tumor of the stomach in a subject comprising the steps of:

(a) providing a composition containing the polypeptide chosen from among any of 7-8, 21, 28-29 or an active fragment thereof, and a pharmaceutically acceptable carrier; and

(b) administering the composition to the subject.

[0079] 56. A method of treatment of a tumor of the bladder in a subject comprising the steps of:

(a) providing a composition containing the polypeptide chosen from among any of 7-8, 21, 28-29 or an active fragment thereof, and a pharmaceutically acceptable carrier; and

(b) administering the composition to the subject.

[0080] 57. A method of treatment of skin cancer in a subject comprising the steps of:

(a) providing a composition containing the polypeptide chosen from among any of 7-8, 21, 28-29 or an active fragment thereof, and a pharmaceutically acceptable carrier; and

(b) administering the composition to the subject.

[0081] 58. Use of the polypeptide chosen from among any of 7-8, 21, 28-29 as a target for screening for a modulator.

[0082] 59. The use of 58, wherein the modulator is a small molecule drug.

[0083] 60. The use of 58, wherein the modulator is an antibody.

[0084] 61. A method of stimulating immune cell proliferation comprising the steps of:

(a) providing a composition comprising a substantially pure polypeptide chosen from among any of SEQ ID NOs: 6-10 and 15; and

(b) contacting one or more immune cell with the polypeptide.

[0085] 62. The method of 61, wherein the immune cell is a monocyte, a lymphocyte, a macrophage, or a peripheral blood mononuclear cell.

[0086] 63. The method of 61, wherein the polypeptide is encoded by a nucleic acid molecule and the nucleic acid molecule comprises a nucleotide sequence chosen from among any of SEQ ID NOs. 1-5, 11-14, and 16.

[0087] 64. A method of stimulating an immune response in a subject, comprising the steps of:

[0088] (a) providing a composition comprising a substantially pure polypeptide chosen from among any of SEQ ID NOs. 6-10 and 15; and

[0089] (b) administering the composition to the subject.

[0090] 65. The method of 64, wherein the step of administering the polypeptide to the subject comprises administering the polypeptide locally or systemically.

[0091] 66. The method of 64, wherein the immune response is mediated by monocytes, lymphocytes, macrophages, or PBMCs.

- [0092] 67. The method of 64, wherein the polypeptide is encoded by a nucleic acid molecule and the nucleic acid molecule comprises a nucleotide sequence chosen from among any of SEQ ID NOs. 1-5, 11-14, and 16.
- [0093] 68. A method for increasing immune cells in a subject after cancer therapy, comprising the steps of:
- [0094] (a) providing a composition comprising a substantially pure polypeptide chosen from among any of SEQ ID NOs. 6-10 and 15; and
- [0095] (b) administering the composition to the subject.
- [0096] 69. The method of 68 where in the immune cells are chosen from among monocytes, lymphocytes, macrophages and PBMCs
- [0097] 70. The method 68, wherein the cancer therapy is chosen from chemotherapy and radiation therapy.
- [0098] 71. The method of 68, wherein the polypeptide is administered after bone marrow transplant.
- [0099] 72. The method of 68, wherein the polypeptide is encoded by a nucleic acid molecule and the nucleic acid molecule comprises a nucleotide sequence chosen from among any of SEQ ID NOs. 1-5, 11-14, and 16.
- [00100] 73. A method for treating or preventing an infection in a subject, comprising the steps of:
- (a) providing a composition comprising a substantially pure polypeptide chosen from among any of SEQ ID NOs. 6-10 and 15; and
- (b) administering the composition to the subject.
- [00101] 74. The method of 73, wherein the infection is chosen from among any of a bacterial infection, a mycoplasma infection, a fungal infection, and a viral infection.
- [00102] 75. The method of 73, wherein the step of administering the composition to the subject comprises administering the composition locally or systemically.
- [00103] 76. The method of 73, wherein the polypeptide is encoded by a nucleic acid molecule and the nucleic acid molecule comprises a nucleotide sequence chosen from among any of SEQ ID NOs. 1-5, 11-14, and 16.
- [00104] 77. A method of modulating an immune response in a subject, comprising the steps of:

(a) providing a modulator of a polypeptide chosen from among any of SEQ ID NOs. 6-10 and 15; and

(b) administering the modulator to the subject.

[00105] 78. The method of 77, wherein the modulator is an antibody.

[00106] 79. The method of 78, wherein the antibody is chosen from among any of a monoclonal antibody, a polyclonal antibody, a cdr fragment, a framework fragment, a single chain antibody, and an active fragment of an antibody.

[00107] 80. The method of 77, wherein the modulation of immune response is suppression of inflammation.

[00108] 81. The method of 77, wherein the modulation of immune response is suppression of autoimmune diseases.

[00109] 82. The method of 77, wherein the modulation of immune response is chosen from treatment of rheumatoid arthritis, osteoarthritis, psoriasis, inflammatory bowel disease, multiple sclerosis, myocardial infarction, stroke, fluminant liver failure.

[00110] 83. A method of enhancing immune response to a vaccine in a subject comprising:

(a) providing a polypeptide composition comprising a substantially purified polypeptide chosen from among SEQ ID NOs. 6-10 and 15;  
(b) providing a vaccine composition; and  
(c) administering the polypeptide composition and the vaccine composition to the subject.

[00111] 84. The method of 83, wherein the polypeptide composition is administered to the subject prior to administering the vaccine composition.

[00112] 85. The method of 83, wherein the polypeptide composition is administered to the subject after administering the vaccine composition.

[00113] 86. The method of 83, wherein the polypeptide composition is administered to the subject with the vaccine composition substantially contemporaneously.

#### DESCRIPTION OF THE FIGURES

[00114] Figure 1 shows the polypeptide alignment between a first newly identified IL-24 splice variant and a know IL-24 variant.



- [00115] Figure 2 shows the polypeptide alignment between a second newly identified IL-24 splice variant and a known IL-24 variant.
- [00116] Figure 3 shows the alignment between the two newly identified IL-24 splice variants.
- [00117] Figure 4 shows the polypeptide alignment between the newly identified Apo2 variant and a known variant.
- [00118] Appendix A illustrates the nucleic acid sequence and polypeptide sequence of the APo2 splice variant of the invention.
- [00119] Appendix B illustrates the nucleic acid sequence and polypeptide sequence of an IL-24 splice variant of the invention.
- [00120] Appendix C illustrates the nucleic acid sequence and polypeptide sequence of a known IL-24 variant.
- [00121] Appendix D illustrates the nucleic acid sequence and polypeptide sequence of another IL-24 splice variant of the invention.
- [00122] Appendix E illustrates the nucleic acid sequence and polypeptide sequence of an exon fragment from a known IL-24 molecule.
- [00123] Appendix F illustrates the nucleic acid sequence and polypeptide sequence of another exon fragment from a known IL-24 molecule.
- [00124] Table 1 (SEQ ID NO. Table): column 1 shows an internal designation ID number; column 2 shows the nucleotide sequence ID number for the open reading frame ("ORF") for the nucleic acid sequence; column 3 shows the amino acid sequence ID number for the polypeptide sequence; column 4 shows the nucleotide sequence ID number for the entire nucleic acid sequence; column 5 shows the polypeptide ID number of the source clone or sequence; column 6 shows the classification/gene family.
- [00125] Table 2 shows the public annotation of the polypeptide sequences (from Appendices A-F): column 1 shows an internal designation ID number of the polypeptide; column 2 shows the source ID number of the polypeptide; column 3 shows the predicted length of the polypeptide; column 4 shows the public ID number of a best hit found in the public database NR; column 5 shows the annotation of the ID number set forth in column 4; column 6 shows the percent identity between the polypeptide and the sequence set forth in column 4; column 7 shows the length of the match between the polypeptide and the

sequence shown in column 4; column 8 shows the public ID number of a best hit found in the public database NR (human only); column 9 shows the annotation of the ID number shown in column 4 (human only); column 10 shows the percent identity between the polypeptide and the sequence shown in column 4 (human only); column 11 shows the length of the match between the polypeptide and the sequence shown in column 4 (human only).

[00126] Table 3 shows pfam, secreted and transmembrane data, and other information about the polypeptides of the invention: column 1 shows an internal designation ID number of the polypeptide; column 2 shows the source ID number of the polypeptide; column 3 shows the cluster ID number of the polypeptide; column 4 shows the classification of the polypeptide; column 5 shows the predicted protein length; column 6 shows an internal parameter “the treevote”; column 7 shows the mature protein coordinates; column 8 shows the alternate mature protein coordinates; column 9 shows the signal peptide coordinates; column 10 shows the number of transmembrane domains; column 11 shows the coordinates of transmembrane domains; column 12 shows the coordinates of non-transmembrane domains; column 13 shows the names of pfam domains within the polypeptide.

[00127] Table 4 shows the coordinates of the predicted pfam domains in the Apo2 polypeptides: column 1 shows an internal designation ID number of the polypeptide; column 2 shows the source ID number of the polypeptide; column 3 shows the name of the pfam domain; column 4 shows the start and stop coordinates of the pfam domain in the polypeptide.

#### DETAILED DESCRIPTION OF THE INVENTION

[00128] The terms used herein have their ordinary meaning and the meanings given them specifically below.

[00129] By “nucleotide sequence” of a nucleic acid molecule or polynucleotide is intended, for a DNA molecule or polynucleotide, a sequence of deoxyribonucleotides, and for an RNA molecule or polynucleotide, the corresponding sequence of ribonucleotides (A, G, C and U), where each thymidine deoxyribonucleotide (T) in the specified deoxyribonucleotide sequence is replaced by the ribonucleotide uridine (U). A “nucleic acid” molecule can include both double- and single-stranded sequences and refers to, but is not limited to,

cDNA from viral, procaryotic or eucaryotic mRNA, genomic DNA sequences from viral (e.g. DNA viruses and retroviruses) or procaryotic DNA, and especially synthetic DNA sequences. The term also captures sequences that include any of the known base analogs of DNA and RNA.

[00130] By “isolated” nucleic acid molecule(s) is intended a nucleic acid molecule, DNA or RNA, which has been removed from its native environment. For example, recombinant DNA molecules contained in a vector are considered isolated for the purposes of the present invention. Further examples of isolated DNA molecules include recombinant DNA molecules maintained in heterologous host cells or purified (partially or substantially) DNA molecules in solution. Isolated RNA molecules include in vivo or in vitro RNA transcripts of the DNA molecules of the present invention. Isolated nucleic acid molecules according to the present invention further include such molecules produced synthetically.

[00131] A “modulator” of the polypeptides or polynucleotides or an “agent” herein is an agonist or antagonist that interferes with the binding or activity of such polypeptides or polynucleotides. Such modulators or agents include, for example, polypeptide variants, whether agonist or antagonist; antibodies, whether agonist or antagonist; soluble receptors, usually antagonists; small molecule drugs, whether agonist or antagonist; RNAi, usually an antagonist; antisense molecules, usually an antagonist; and ribozymes, usually an antagonist. In some embodiments, an agent is a subject polypeptide, where the subject polypeptide itself is administered to an individual. In some embodiments, an agent is an antibody specific for a subject “target” polypeptide. In some embodiments, an agent is a chemical compound such as a small molecule that may be useful as an orally available drug. Such modulation includes the recruitment of other molecules that directly effect the modulation. For example, an antibody that modulates the activity of a subject polypeptide that is a receptor on a cell surface may bind to the receptor and fix complement, activating the complement cascade and resulting in lysis of the cell. An agent which modulates a biological activity of a subject polypeptide or polynucleotide increases or decreases the activity or binding at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 50%, at least about 80%, or at least about 2-fold, at least about 5-fold, or at least about 10-fold or more when compared to a suitable control.

[00132] “Modulating a level of active subject polypeptide” includes increasing or decreasing activity of a subject polypeptide; increasing or decreasing a level of active polypeptide protein; and increasing or decreasing a level of mRNA encoding active subject polypeptide.

[00133] “Treatment,” as used herein, covers any treatment of a condition or disease in a mammal, including a human, and includes preventing the condition or disease from occurring or recurring in a subject who may be predisposed to the condition or disease but has not yet been diagnosed as having it, inhibiting the condition or disease, i.e., arresting its development, or relieving the condition or disease, i.e., causing regression of the condition or disease, or restoring or repairing a lost, missing or defective function, or stimulating an inefficient process.

[00134] In the context of cancer, the term “treating” includes any or all of: preventing growth of tumor cells or cancer cells, preventing replication of tumor cells or cancer cells, lessening of overall tumor burden and ameliorating one or more symptoms associated with the disease.

[00135] In the context of an autoimmune disease, the term “treating” includes any or all of: preventing replication of cells associated with an autoimmune disease state including, but not limited to, cells capable of producing an autoimmune antibody, lessening the autoimmune-antibody burden and ameliorating one or more symptoms of an autoimmune disease.

[00136] In the context of an infectious disease, the term “treating” includes any or all of preventing the growth, multiplication or replication of the pathogen that causes the infectious disease and ameliorating one or more symptoms of an infectious disease.

[00137] In the context of an ischemic disease, the term “treating” includes any or all of preventing the growth, multiplication or replication of the pathogen that causes the ischemic disease and ameliorating one or more symptoms of an ischemic disease.

[00138] “Operably linked” refers to an arrangement of elements wherein the components so described are configured so as to perform their desired function. Thus, a given promoter operably linked to a coding sequence is capable of effecting the expression of the coding sequence when the proper transcription factors, etc., are present. The promoter need not be contiguous with the coding sequence, so long as it functions to direct the expression thereof. Thus, for example, intervening untranslated yet transcribed sequences can be present

between the promoter sequence and the coding sequence, as can translated introns, and the promoter sequence can still be considered "operably linked" to the coding sequence.

[00139] "Recombinant" as used herein to describe a nucleic acid molecule means a polynucleotide of genomic, cDNA, viral, semisynthetic, or synthetic origin which, by virtue of its origin or manipulation is not associated with all or a portion of the polynucleotide with which it is associated in nature. The term "recombinant" as used with respect to a protein or polypeptide means a polypeptide produced by expression of a recombinant polynucleotide.

[00140] A "control element" refers to a polynucleotide sequence which aids in the expression of a coding sequence to which it is linked. The term includes promoters, transcription termination sequences, upstream regulatory domains, polyadenylation signals, and when appropriate, leader sequences and enhancers, which collectively provide for the transcription and translation of a coding sequence in a host cell.

[00141] A "promoter" as used herein is a DNA regulatory region capable of binding RNA polymerase in a mammalian cell and initiating transcription of a downstream (3' direction) coding sequence operably linked thereto. For purposes of the present invention, a promoter sequence includes the minimum number of bases or elements necessary to initiate transcription of a gene of interest at levels detectable above background. Within the promoter sequence is a transcription initiation site, as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase. Eucaryotic promoters will often, but not always, contain "TATA" boxes and "CAT" boxes. Promoters further include those that are naturally contiguous to a nucleic acid molecule and those that are not naturally contiguous to a nucleic acid molecule. Additionally, a promoter includes inducible promoters, conditionally active promoters, such as a cre-lox promoter, constitutive promoters and a tissue specific promoters.

[00142] By "selectable marker" is meant a gene which confers a phenotype on a cell expressing the marker, such that the cell can be identified under appropriate conditions. Generally, a selectable marker allows selection of transformed cells based on their ability to thrive in the presence or absence of a chemical or other agent that inhibits an essential cell function. Suitable markers, therefore, include genes coding for proteins which confer drug resistance or sensitivity thereto, impart color to, or change the antigenic characteristics of

those cells transfected with a molecule encoding the selectable marker, when the cells are grown in an appropriate selective medium. For example, selectable markers include: cytotoxic markers and drug resistance markers, whereby cells are selected by their ability to grow on media containing one or more of the cytotoxins or drugs; auxotrophic markers by which cells are selected by their ability to grow on defined media with or without particular nutrients or supplements, such as thymidine and hypoxanthine; metabolic markers by which cells are selected for, e.g., their ability to grow on defined media containing the appropriate sugar as the sole carbon source, or markers which confer the ability of cells to form colored colonies on chromogenic substrates or cause cells to fluoresce.

[00143] “Transformation,” as used herein, refers to the insertion of an exogenous polynucleotide into a host cell, irrespective of the method used for insertion: for example, transformation by direct uptake, transfection, infection, and the like. For particular methods of transfection, see further below. The exogenous polynucleotide may be maintained as a nonintegrated vector, for example, an episome, or alternatively, may be integrated into the host genome.

[00144] The terms “polypeptide” and “protein” refer to a polymer of amino acid residues and are not limited to a minimum length of the product. Thus, peptides, oligopeptides, dimers, multimers, and the like, are included within the definition. Both full-length proteins and fragments thereof are encompassed by the definition. The terms also include post-expression modifications of the polypeptide, for example, glycosylation, acetylation, phosphorylation and the like. Furthermore, for purposes of the present invention, a “polypeptide” refers to a protein which includes modifications, such as deletions, additions and substitutions (generally conservative in nature), to the native sequence, as long as the protein maintains the desired activity. These modifications may be deliberate, as through site-directed mutagenesis, or may be accidental, such as through mutations of hosts which produce the proteins or errors due to PCR amplification.

[00145] A “gene,” for the purposes of the present disclosure, includes a DNA region encoding a gene product, as well as all DNA regions which regulate the production of the gene product, whether or not such regulatory sequences are adjacent to coding and/or transcribed sequences. Accordingly, a gene includes, but is not necessarily limited to, promoter sequences, terminators, translational regulatory sequences such as ribosome

binding sites and internal ribosome entry sites, enhancers, silencers, insulators, boundary elements, replication origins, matrix attachment sites and locus control regions.

[00146] “Gene expression” refers to the conversion of the information, contained in a gene, into a gene product. A gene product can be the direct transcriptional product of a gene (*e.g.*, mRNA, tRNA, rRNA, antisense RNA, ribozyme, structural RNA or any other type of RNA) or a protein produced by translation of an mRNA. Gene products also include RNAs which are modified, by processes such as capping, polyadenylation, methylation, and editing, and proteins modified by, for example, methylation, acetylation, phosphorylation, ubiquitination, ADP-ribosylation, myristilation, and glycosylation.

[00147] A “coding sequence” or a sequence which “encodes” a selected polypeptide, is a nucleic acid molecule which is transcribed (in the case of DNA) and translated (in the case of mRNA) into a polypeptide *in vivo* when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxy) terminus. A coding sequence can include, but is not limited to, cDNA from viral, procaryotic or eucaryotic mRNA, genomic DNA sequences from viral (*e.g.* DNA viruses and retroviruses) or procaryotic DNA, and especially synthetic DNA sequences. A transcription termination sequence may be located 3' to the coding sequence.

[00148] As used herein, the term “antibody” encompasses polyclonal and monoclonal antibody preparations, as well as preparations including hybrid antibodies, altered antibodies, chimeric antibodies and, humanized antibodies, as well as: hybrid (chimeric) antibody molecules (see, for example, Winter et al., *Nature* 349:293-299 (1991)); and U.S. Patent No. 4,816,567); F(ab')<sub>2</sub> and F(ab) fragments; Fv molecules (noncovalent heterodimers, see, for example, Inbar et al., *Proc Natl Acad Sci USA* 69:2659-2662 (1972)); and Ehrlich *et al.* (1980) *Biochem* 19:4091-4096); single-chain Fv molecules (sFv) (*see, e.g.*, Huston et al., *Proc Natl Acad Sci USA* 85:5879-5883 (1980)); dimeric and trimeric antibody fragment constructs; minibodies (*see, e.g.*, Pack et al., *Biochem* 31:1579-1584 (1992); Cumber et al., *J. Immunology* 149B:120-126 (1992)); humanized antibody molecules (*see, e.g.*, Riechmann et al., *Nature* 332:323-327 (1988); Verhoeyan et al., *Science* 239:1534-1536 (1988)); and, any functional fragments obtained from such molecules, wherein such fragments retain specific-binding.

[00149] As used herein, the term “monoclonal antibody” refers to an antibody composition having a homogeneous antibody population. The term is not limited regarding the species or source of the antibody, nor is it intended to be limited by the manner in which it is made. The term encompasses whole immunoglobulins.

[00150] Methods of making polyclonal and monoclonal antibodies are known in the art. Polyclonal antibodies are generated by immunizing a suitable animal, such as a mouse, rat, rabbit, sheep or goat, with an antigen of interest, such as a stem cell transformed with a gene encoding an antigen. In order to enhance immunogenicity, the antigen can be linked to a carrier prior to immunization. Suitable carriers are typically large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, lipid aggregates (such as oil droplets or liposomes), and inactive virus particles. Such carriers are well known to those of ordinary skill in the art. Furthermore, the antigen may be conjugated to a bacterial toxoid, such as toxoid from diphtheria, tetanus, cholera, etc., in order to enhance the immunogenicity thereof.

[00151] In addition, techniques developed for the production of “chimeric antibodies” (Morrison et al., PNAS, 81:851-855 (1984); Neuberger et al., *Nature*, 312:604-608 (1984); Takeda et al., *Nature*, 314:452-454 (1985)) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region, for example, humanized antibodies, and insertion/deletions relating to cdr and framework regions.

[00152] The term “subject,” as used herein, refers to a subject, such as a living animal, including a human and a non-human animal. The subject is an organism possessing immune cells capable of responding to antigenic stimulation and stimulatory and inhibitory signaling transduction through cell surface receptor binding. In preferred embodiments, the subject is a mammal, including humans and non-human mammals such as dogs, cats, pigs, cows, sheep, goats, horses, rats, and mice. In the most preferred embodiment, the subject is



a human. The term “subject” does not preclude individuals that are entirely normal with respect to a disease, or normal in all respects.

**[00153]** By “isolated” is meant, when referring to a polynucleotide or polypeptide of the invention, that the indicated molecule is substantially separated, e.g., from the whole organism in which the molecule is found or from the cell culture in which the antibody is produced, or is present in the substantial absence of other biological macromolecules of the same type.

**[00154]** The term “specifically binds,” refers to high avidity and/or high affinity binding of an antibody to a specific polypeptide, or more accurately, to an epitope of an antigen. Antibody specifically binding to such epitope on the receptor can be stronger than binding of the same antibody to any other epitopes, particularly other epitopes that can be present in molecules in association with, or in the same sample as the receptor of interest. For example, when an antibody binds more strongly to one epitope than to another, adjusting the binding conditions can result in antibody binding almost exclusively to the specific epitope and not to any other epitopes on the same polypeptide, and not to any other polypeptide, which does not comprise the epitope.

**[00155]** The term “agonist,” in the context of an antibody, refers to a molecule that mimics, enhances, stimulates or activates the function of a molecule with which the agonist interacts. Agonists include, but are not limited to, analogues and fragments thereof.

**[00156]** The term “antagonist,” in the context of an antibody, refers to a molecule that competes, inhibits or interferes with the activity of a molecule with which the antagonist interacts. For example, an antagonist antibody can bind to the receptor, but does not induce an active response. Antagonists include, but are not limited to, analogues and fragments thereof.

**[00157]** By “fragment” is intended a polypeptide consisting of only a part of the intact full-length polypeptide sequence and structure. The fragment can include a C-terminal deletion an N-terminal deletion, and/or an internal deletion of the native polypeptide. A fragment of a protein will generally include at least about 5-10 contiguous amino acid residues of the full-length molecule, preferably at least about 15-25 contiguous amino acid residues of the full-length molecule, and most preferably at least about 20-50 or more contiguous amino

acid residues of the full-length molecule, or any integer between 5 amino acids and the full-length sequence.

[00158] As used herein, the phrase “pharmaceutically acceptable carrier” is intended to include substances that can be co-administered with the compositions of the invention that allows the composition or active molecule therein to perform its intended function. Examples of such carriers include solutions, solvents, buffers, adjuvants, dispersion media, delay agents, emulsions and the like. Further, any other conventional carrier, suitable for use with the described antibodies fall within the scope of the instant invention, such as, for example, phosphate buffered saline.

### **Nucleic Acid and Polypeptides of the Invention**

[00159] The present invention provides nucleic acid molecules containing a polynucleotide encoding a newly identified Apo2 variant polypeptide and two newly identified IL-24 variant polypeptides having the amino acid sequences as shown in Appendices A-F (SEQ ID NOs. 15, 6, and 8). The isolated Apo2 and IL-24 variants of the invention were identified in bioinformatics analysis of all of the Apo2 and IL-24 clones that were identified. Specifically, the Apo2 variant polypeptide is structurally related to members of the tumor necrosis factor (“TNF”) gene super family, and contains an open reading frame encoding a polypeptide of 235 amino acids (SEQ ID NO.15). The IL-24 variant polypeptides are related to members of the IL-10 cytokine family. The first IL-24 variant contains an open reading frame encoding a polypeptide of 179 amino acids (SEQ ID NO.6), and the second IL-24 variant contains an open reading frame encoding a polypeptide of 126 amino acids (SEQ ID NO.8). IL-24 has been found to predominantly expressed in tissues, such as, trachea, bile duct, cerebral cortex, cortex of the occipital lobe, smooth muscle, meniscus of the joint, brain and tonsil.

[00160] Fragments of the full length Apo2 and IL-24 variants may be used as hybridization probes for cDNA libraries to isolate the full length gene and to isolate other genes which have a high sequence similarity to the gene or similar biological activity. Probes of this type preferably have at least 30 bases and may contain, for example, 50 or more bases. The probe may also be used to identify a cDNA clone corresponding to a full length transcript and a genomic clone or clones that contain the complete Apo2 and IL-24 genes including

regulatory and promotor regions, exons, and introns. An example of a screen contains isolating the coding regions of the Apo2 and IL-24 genes by using the known nucleic acid sequence to synthesize an oligonucleotide probe. Labeled oligonucleotides having a sequence complementary to that of the gene of the present invention are used to screen a library of human cDNA, genomic DNA or mRNA to determine which members of the library the probe hybridizes to.

[00161] The present invention further relates to polynucleotides which hybridize to the hereinabove-described sequences if there is at least 91%, preferably at least 92%, and more preferably at least 95% identity between the sequences. The present invention particularly relates to polynucleotides which hybridize under stringent conditions to the hereinabove-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences. For example, overnight incubation at 42°C in a solution containing: 50% formamide, 5x SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 µg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C.

[00162] The polynucleotides which hybridize to the hereinabove described polynucleotides in a preferred embodiment encode polypeptides which either retain substantially the same biological function or activity as the mature polypeptide.

[00163] Alternatively, the polynucleotide may have at least 20 bases, preferably 30 bases, and more preferably at least 50 bases which hybridize to a polynucleotide of the present invention and which has an identity thereto, as hereinabove described, and which may or may not retain activity.

[00164] Thus, the present invention is directed to polynucleotides having at least a 70% identity, preferably at least 90% and more preferably at least a 95% identity to a polynucleotide which encodes the polypeptides set forth in Appendices A-F as well as fragments thereof, which fragments have at least 30 bases and preferably at least 50 bases and to polypeptides encoded by such polynucleotides.

[00165] Using the information provided herein, such as the nucleotide sequences set forth in Appendices A-F, nucleic acid molecules of the present invention encoding an Apo2 and IL-24 polypeptide may be obtained using standard cloning and screening procedures, such as

those for cloning cDNAs using mRNA as starting material. Nucleic acids of the invention are useful as hybridization probes for differential identification of the tissue(s) or cell type(s) present in a biological sample. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of tissue(s) or cell type(s).

### **Variant and Mutant Polynucleotides**

**[00166]** The present invention further relates to variants of the nucleic acid molecules of the present invention, which encode portions, analogs or derivatives of the Apo2 and IL-24 molecules. Variants may occur naturally, such as a natural allelic variant. By an “allelic variant” is intended one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. Genes II, Lewin, B., ed., John Wiley & Sons, New York (1985). Non-naturally occurring variants may be produced using art-known mutagenesis techniques.

**[00167]** Such variants include those produced by nucleotide substitutions, deletions or additions. The substitutions, deletions or additions may involve one or more nucleotides. The variants may be altered in coding regions, non-coding regions, or both. Alterations in the coding regions may produce conservative or non-conservative amino acid substitutions, deletions or additions. Especially preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of the described Apo2 and IL-24 proteins or portions thereof. Also especially preferred in this regard are conservative substitutions.

**[00168]** Most highly preferred are nucleic acid molecules encoding the mature proteins having the amino acid sequences as shown, for example, in Appendices A-F, or the Apo2 and IL-24 polypeptides encoded by the nucleic acid molecules of Appendices A-F. Further embodiments include an isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 93% identical, and more preferably at least 95%, 96%, 97%, 98% or 99% identical to a polynucleotide selected from the group consisting of: (a) a nucleotide sequence encoding an Apo2 or IL-24 polypeptide having the complete amino acid sequences as set forth in Appendices A-F (SEQ ID NOs. 15, 6, and 8); (b) a biologically active fragment of such.

[00169] By a polynucleotide having a nucleotide sequence at least, for example, 95% “identical” to a reference nucleotide sequence encoding an Apo2 or IL-24 polypeptide is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence encoding the Apo2 or IL-24 polypeptides. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

[00170] As a practical matter, whether any particular nucleic acid molecule is at least 93%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the nucleotide sequences set forth in Appendices A-F or to the nucleotides sequence of the deposited cDNA clones can be determined conventionally using known computer programs such as the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, Wis. 53711). Bestfit uses the local homology algorithm of Smith and Waterman, *Advances in Applied Mathematics* 2:482-489 (1981), to find the best segment of homology between two sequences. When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference nucleotide sequence and that gaps in homology of up to 5% of the total number of nucleotides in the reference sequence are allowed.

[00171] The present application is directed to nucleic acid molecules at least 93%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequences set forth in Appendices A-F, irrespective of whether they encode a polypeptide having Apo2 or IL-24 activity. This is because even where a particular nucleic acid molecule does not encode a polypeptide having Apo2 or IL-24 activity, one of skill in the art would still know how to use the

nucleic acid molecules, for instance, as a hybridization probe or a polymerase chain reaction (PCR) primer. Uses of the nucleic acid molecules of the present invention that do not encode a polypeptide having Apo2 or IL-24 activity include, inter alia, (1) isolating the Apo2 or IL-24 gene or allelic variants thereof in a cDNA library; (2) in situ hybridization (e.g., "FISH") to metaphase chromosomal spreads to provide precise chromosomal location of the Apo2 or IL-24 genes, as described in Verna et al., *Human Chromosomes: A Manual of Basic Techniques*, Pergamon Press, New York (1988); and Northern blot analysis for detecting Apo2 or IL-24 mRNA expression in specific tissues.

[00172] Preferred, however, are nucleic acid molecules having sequences at least 93%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequence shown in Appendices A-F which do, in fact, encode a polypeptide having Apo2 or IL-24 polypeptide activity. By "a polypeptide having Apo2 or IL-24 activity" is intended polypeptides exhibiting activity similar, but not necessarily identical, to an activity of the Apo2 and IL-24 polypeptides of the invention, as measured in a particular biological assay. For example, the Apo2 and IL-24 polypeptides of the present invention may stimulate proliferation of various mammalian cells.

[00173] Of course, due to the degeneracy of the genetic code, one of ordinary skill in the art will immediately recognize that a large number of the nucleic acid molecules having a sequence at least 93%, 95%, 96%, 97%, 98%, or 99% identical to the nucleic acid sequence of the deposited cDNA or the nucleic acid sequences set forth in Appendices A-F, will encode a polypeptide "having Apo2 or IL-24 polypeptide activity." In fact, since degenerate variants of these nucleotide sequences all encode the same polypeptide, this will be clear to the skilled artisan even without performing the above described comparison assay. It will be further recognized in the art that, for such nucleic acid molecules that are not degenerate variants, a reasonable number will also encode a polypeptide having Apo2 or IL-24 polypeptide activity. This is because the skilled artisan is fully aware of amino acid substitutions that are either less likely or not likely to significantly effect protein function (e.g., replacing one aliphatic amino acid with a second aliphatic amino acid), as further described below.

## Vectors and Host Cells

[00174] The present invention also relates to vectors which include the isolated nucleic acid molecules of the present invention, host cells which are genetically engineered with the recombinant vectors, and the production of Apo2 and IL-24 polypeptides or fragments thereof by recombinant techniques. The vector may be, for example, a phage, plasmid, viral or retroviral vector. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host cells.

[00175] The polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

[00176] The DNA insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the E. coli lac, trp, phoA and tac promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination and, in the transcribed region, a ribosome binding site for translation. The coding portion of the transcripts expressed by the constructs will preferably include a translation initiating codon at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

[00177] As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase, G418 or neomycin resistance for eukaryotic cell culture and tetracycline, kanamycin or ampicillin resistance genes for culturing in E. coli and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as E. coli, Streptomyces and Salmonella typhimurium cells; fungal cells, such as yeast cells; insect cells such as Drosophila S2 and Spodoptera Sf9 cells; animal cells such as CHO, COS, 293 and Bowes melanoma cells; and

plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

[00178] Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from QIAGEN, Inc., supra; pBS vectors, Phagescript vectors, Bluescript vectors, pNH8A, pNH6a, pNH18A, pNH46A, available from Stratagene; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Other suitable vectors will be readily apparent to the skilled artisan.

[00179] Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al., Basic Methods In Molecular Biology (1986).

[00180] The polypeptides may be expressed in a modified form, such as a fusion protein, and may include not only secretion signals, but also additional heterologous functional regions. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence in the host cell, during purification, or during subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide.

[00181] The addition of peptide moieties to polypeptides to engender secretion or excretion, to improve stability and to facilitate purification, among others are familiar and routine techniques in the art. A preferred fusion protein comprises a heterologous region from immunoglobulin that is useful to stabilize and purify proteins. For example, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins containing various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is thoroughly advantageous for use in therapy and diagnosis and thus results, for example, in improved pharmacokinetic properties (EP-A 0232 262). On the other hand, for some uses it would be desirable to be able to delete the Fc part after the fusion protein has been expressed, detected and purified



in the advantageous manner described. This is the case when Fc portion proves to be a hindrance to use in therapy and diagnosis, for example when the fusion protein is to be used as antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. See, Bennett et al., *J. Molecular Recognition*, 8:52-58 (1995) and Johanson et al, *J. Biol. Chem.*, 270:9459-9471 (1995).

[00182] The Apo2 and IL-24 polypeptides can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification. Polypeptides of the present invention include: products purified from natural sources, including bodily fluids, tissues and cells, whether directly isolated or cultured; products of chemical synthetic procedures; and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes. Thus, it is well known in the art that the N-terminal methionine encoded by the translation initiation codon generally is removed with high efficiency from any protein after translation in all eukaryotic cells. While the N-terminal methionine on most proteins also is efficiently removed in most prokaryotes, for some proteins this prokaryotic removal process is inefficient, depending on the nature of the amino acid to which the N-terminal methionine is covalently linked.

### **Polypeptides and Fragments**

[00183] The invention further provides an isolated Apo2 and IL-24 polypeptide containing the amino acid sequences encoded by the nucleotide sequences set forth in Appendices A-F, or the amino acid sequences set forth in Appendices A-F, or a peptide or polypeptide comprising a portion of the polypeptide.

### **Variant and Mutant Polypeptides**

[00184] To improve or alter the characteristics of Apo2 and IL-24 polypeptides of the invention, protein engineering may be employed. Recombinant DNA technology known to those skilled in the art can be used to create novel mutant proteins or “muteins” including single or multiple amino acid substitutions, deletions, additions or fusion proteins. Such modified polypeptides can show, e.g., enhanced activity or increased stability. In addition, they may be purified in higher yields and show better solubility than the corresponding natural polypeptide, at least under certain purification and storage conditions.

### **N-Terminal and C-Terminal Deletion Mutants**

[00185] For instance, for many proteins, including the extracellular domain of a membrane associated protein or the mature form(s) of a secreted protein, it is known in the art that one or more amino acids may be deleted from the N-terminus or C-terminus without substantial loss of biological function. For instance, Ron et al., *J. Biol. Chem.*, 268:2984-2988 (1993), reported modified KGF proteins that had heparin binding activity even if 3, 8, or 27 amino-terminal amino acid residues were missing.

[00186] However, even if deletion of one or more amino acids from the N-terminus of a protein results in modification or loss of one or more biological functions of the protein, other biological activities may still be retained. Thus, the ability of the shortened protein to induce and/or bind to antibodies which recognize the complete or mature form of the protein generally will be retained when less than the majority of the residues of the complete or mature protein are removed from the N-terminus. Whether a particular polypeptide lacking N-terminal residues of a complete protein retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art.

[00187] Accordingly, the present invention further provides polypeptides having one or more residues deleted from the amino terminus of the amino acid sequences of the Apo2 and IL-24 molecules as shown in Appendices A-F.

[00188] Similarly, many examples of biologically functional C-terminal deletion muteins are known. For instance, interferon gamma shows up to ten times higher activities by deleting 8-

10 amino acid residues from the carboxy terminus of the protein, see, for example, Dobeli et al., *J. Biotechnology*, 7:199-216 (1988).

[00189] However, even if deletion of one or more amino acids from the C-terminus of a protein results in modification or loss of one or more biological functions of the protein, other biological activities may still be retained. Thus, the ability of the shortened protein to induce and/or bind to antibodies which recognize the complete or mature form of the protein generally will be retained when less than the majority of the residues of the complete or mature protein are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete protein retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art.

#### Other Mutants

[00190] In addition to terminal deletion forms of the protein discussed above, it also will be recognized by one of ordinary skill in the art that some amino acid sequences of the Apo2 and IL-24 polypeptides can be varied without significant effect of the structure or function of the protein. If such differences in sequence are contemplated, it should be remembered that there will be critical areas on the protein which determine activity.

[00191] Thus, the invention further includes variations of the Apo2 and IL-24 polypeptides which show substantial Apo2 or IL-24 polypeptide activity or which include regions of the Apo2 or IL-24 proteins such as the protein portions discussed below. Such mutants include deletions, insertions, inversions, repeats, and type substitutions selected according to general rules known in the art so as to have little effect on activity. For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie et al., "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," *Science*, 247:1306-1310 (1990), wherein the authors indicate that there are two main approaches for studying the tolerance of an amino acid sequence to change. The first method relies on the process of evolution, in which mutations are either accepted or rejected by natural selection. The second approach uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene and selections or screens to identify sequences that maintain functionality.

[00192] As the authors state, these studies have revealed that proteins are surprisingly tolerant of amino acid substitutions. The authors further indicate which amino acid changes are likely to be permissive at a certain position of the protein. For example, most buried amino acid residues require nonpolar side chains, whereas few features of surface side chains are generally conserved. Other such phenotypically silent substitutions are described in Bowie, J. U. et al., supra, and the references cited therein. Typically seen as conservative substitutions are the replacements, one for another, among the aliphatic amino acids Ala, Val, Leu and Ile; interchange of the hydroxyl residues Ser and Thr, exchange of the acidic residues Asp and Glu, substitution between the amide residues Asn and Gln, exchange of the basic residues Lys and Arg and replacements among the aromatic residues Phe, Tyr.

[00193] Thus, a fragment, derivative or analog of the polypeptide of Appendix B or the polypeptide encoded by the nucleic acid sequence of Appendix A, may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the above form of the polypeptide, such as an IgG Fc fusion region peptide or leader or secretory sequence or a sequence which is employed for purification of the above form of the polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

[00194] Thus, the Apo2 and IL-24 polypeptides of the present invention may include one or more amino acid substitutions, deletions or additions, either from natural mutations or human manipulation. As indicated, changes are preferably of a minor nature, such as conservative amino acid substitutions that do not significantly affect the folding or activity of the protein as shown below:

Conservative Amino Acid Substitutions.

Aromatic	Phenylalanine
	Tryptophan
	Tyrosine
Hydrophobic	Leucine
	Isoleucine
	Valine
Polar	Glutamine
	Asparagine
Basic	Arginine
	Lysine
	Histidine
Acidic	Aspartic Acid
	Glutamic Acid
Small	Alanine
	Serine
	Threonine
	Methionine
	Glycine

[00195] Amino acids in the Apo2 and IL-24 polypeptides of the present invention that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis, see, for example, Cunningham and Wells, *Science*, 244:1081-1085 (1989). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for biological activity such as receptor binding or in vitro or in vitro proliferative activity.

[00196] Of special interest are substitutions of charged amino acids with other charged or neutral amino acids which may produce proteins with highly desirable improved characteristics, such as less aggregation. Aggregation may not only reduce activity but also be problematic when preparing pharmaceutical formulations, because aggregates can be immunogenic, for example, Pinckard et al., *Clin. Exp. Immunol.*, 2:331-340 (1967);

Robbins et al., *Diabetes*, 36:838-845 (1987); Cleland et al., *Crit. Rev. Therapeutic Drug Carrier Systems*, 10:307-377 (1993).

[00197] Replacement of amino acids can also change the selectivity of the binding of a ligand to cell surface receptors. For example, Ostade et al., *Nature*, 361:266-268 (1993) describes certain mutations resulting in selective binding of TNF- $\alpha$  to only one of the two known types of TNF receptors. Sites that are critical for ligand-receptor binding can also be determined by structural analysis such as crystallization, nuclear magnetic resonance or photoaffinity labeling, for example, Smith et al., *J. Mol. Biol.*, 224:899-904 (1992) and de Vos et al., *Science*, 255:306-312 (1992).

[00198] The polypeptides of the present invention are preferably provided in an isolated form, and preferably are substantially purified. A recombinantly produced version of the herein described Apo2 and IL-24 polypeptides can be substantially purified by the one-step method described in Smith and Johnson, *Gene*, 67:31-40 (1988). Polypeptides of the invention also can be purified from natural or recombinant sources using anti-Apo2 and IL-24 antibodies of the invention in methods which are well known in the art of protein purification.

[00199] Further polypeptides of the present invention include polypeptides which have at least 93% similarity, more preferably at least 95% similarity, and still more preferably at least 96%, 97%, 98% or 99% similarity to those described above. The polypeptides of the invention also contain those which are at least 93% identical, more preferably at least 94% or 95% identical, still more preferably at least 96%, 97%, 98% or 99% identical to the polypeptide encoded by the nucleic acid sequences set forth in Appendices A-F.

[00200] By “% similarity” for two polypeptides is intended a similarity score produced by comparing the amino acid sequences of the two polypeptides using the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, Wis. 53711) and the default settings for determining similarity. Bestfit uses the local homology algorithm of Smith and Waterman, *Advances in Applied Mathematics* 2:482-489, (1981), to find the best segment of similarity between two sequences.

[00201] By a polypeptide having an amino acid sequence at least, for example, 95% “identical” to a reference amino acid sequence of an Apo2 or IL-24 polypeptide is intended

that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of the Apo2 or IL-24 polypeptides. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence: These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

[00202] As a practical matter, whether any particular polypeptide is at least 93%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the amino acid sequence shown in Appendix B or to the polypeptide sequence encoded by the nucleic acid sequence set forth in Appendix A, can be determined conventionally using known computer programs such the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, Wis. 53711). When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference amino acid sequence and that gaps in homology of up to 5% of the total number of amino acid residues in the reference sequence are allowed.

[00203] The polypeptide of the present invention could be used as a molecular weight marker on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art. As described in detail below, the polypeptides of the present invention can also be used to raise polyclonal and monoclonal antibodies, which are useful in assays for detecting Apo2 or IL-24 protein expression as described below or as agonists and antagonists capable of enhancing or inhibiting Apo2 or IL-24 protein function. Further, such polypeptides can be used in the yeast two-hybrid system to "capture" Apo2 or IL-24 protein binding proteins which are also candidate agonists and antagonists according

to the present invention. The yeast two hybrid system is described in Fields and Song, *Nature*, 340:245-246 (1989).

### **Epitope-Bearing Portions**

- [00204] In another aspect, the invention provides a peptide or polypeptide comprising an epitope-bearing portion of a polypeptide of the invention. The epitope of this polypeptide portion is an immunogenic or antigenic epitope of a polypeptide of the invention. An “immunogenic epitope” is defined as a part of a protein that elicits an antibody response when the whole protein is the immunogen. On the other hand, a region of a protein molecule to which an antibody can bind is defined as an “antigenic epitope.” The number of immunogenic epitopes of a protein generally is less than the number of antigenic epitopes. See, for instance, Geysen et al., *Proc. Natl Acad. Sci.*, USA 81:3998-4002 (1983).
- [00205] As to the selection of peptides or polypeptides bearing an antigenic epitope (i.e., that contain a region of a protein molecule to which an antibody can bind), it is well known in that art that relatively short synthetic peptides that mimic part of a protein sequence are routinely capable of eliciting an antiserum that reacts with the partially mimicked protein. See, for instance, Sutcliffe et al., *Science*, 219:660-666 (1983). Peptides capable of eliciting protein-reactive sera are frequently represented in the primary sequence of a protein, can be characterized by a set of simple chemical rules, and are confined neither to immunodominant regions of intact proteins (i.e., immunogenic epitopes) nor to the amino or carboxyl terminals. Antigenic epitope-bearing peptides and polypeptides of the invention are therefore useful to raise antibodies, including monoclonal antibodies, that bind specifically to a polypeptide of the invention. See, for instance, Wilson et al., *Cell*, 37:767-778 (1984).
- [00206] The epitope-bearing peptides and polypeptides of the invention may be produced by any conventional means. See, for example, Houghten, *Proc. Natl. Acad. Sci.*, USA 82:5131-5135 (1985), and U.S. Pat. No. 4,631,211 (1986).
- [00207] Epitope-bearing peptides and polypeptides of the invention are used to induce antibodies according to methods well known in the art. See, for instance, Bittle, F. J. et al, *J. Gen. Virol.*, 66:2347-2354 (1985). Immunogenic epitope-bearing peptides of the invention, i.e., those parts of a protein that elicit an antibody response when the whole protein is the



immunogen, are identified according to methods known in the art. See, for instance, U.S. Pat. No. 5,194,392 (1990) describes a general method of detecting or determining the sequence of monomers (amino acids or other compounds) which is a topological equivalent of the epitope (i.e., a “mimotope”) which is complementary to a particular paratope (antigen binding site) of an antibody of interest. More generally, U.S. Pat. No. 4,433,092 (1989) describes a method of detecting or determining a sequence of monomers which is a topographical equivalent of a ligand which is complementary to the ligand binding site of a particular receptor of interest. Similarly, U.S. Pat. No. 5,480,971 (1996) on Peralkylated Oligopeptide Mixtures discloses linear C1-C7-alkyl peralkylated oligopeptides and sets and libraries of such peptides, as well as methods for using such oligopeptide sets and libraries for determining the sequence of a peralkylated oligopeptide that preferentially binds to an acceptor molecule of interest. Thus, non-peptide analogs of the epitope-bearing peptides of the invention also can be made routinely by these methods.

## **Fusion Proteins**

[00208] As one of skill in the art will appreciate, Apo2 and IL-24 polypeptides of the present invention and the epitope-bearing fragments thereof described above can be combined with parts of the constant domain of immunoglobulins (IgG), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life in vivo. This has been shown, e.g., for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins, for example, EP A 394,827; Traunecker et al., *Nature*, 331:84-86 (1988). Fusion proteins that have a disulfide-linked dimeric structure due to the IgG part can also be more efficient in binding and neutralizing other molecules than the monomeric Apo2 or IL-24 protein or protein fragment alone, for example, Fountoulakis et al., *J. Biochem.*, 270:3958-3964 (1995).

## **Compositions**

[00209] In other aspects, the present invention provides a composition containing an effective amount of a molecule, such as a polypeptide or nucleic acid sequence, of the invention and a pharmaceutically acceptable carrier or vehicle. The compositions are

suitable for veterinary or human administration. The compositions of the present invention can be in any form that allows for the composition to be administered to an animal. For example, the composition can be in the form of a solid, liquid or gas (aerosol). Typical routes of administration include, without limitation, oral, topical, parenteral, sublingual, rectal, vaginal, ocular, and intranasal. Parenteral administration includes subcutaneous injections, intravenous, intramuscular, intrasternal injection or infusion techniques. Preferably, the compositions are administered parenterally. Pharmaceutical compositions of the invention can be formulated so as to allow a molecule of the invention to be bioavailable upon administration of the composition to an animal. Compositions can take the form of one or more dosage units, where for example, a tablet can be a single dosage unit, and a container of a molecule of the invention in aerosol form can hold a plurality of dosage units.

[00210] Materials used in preparing the pharmaceutical compositions can be non-toxic in the amounts used. It will be evident to those of ordinary skill in the art that the optimal dosage of the active ingredient(s) in the pharmaceutical composition will depend on a variety of factors. Relevant factors include, without limitation, the type of animal (e.g., human), the particular form of the molecule of the invention, the manner of administration, and the composition employed.

[00211] The pharmaceutically acceptable carrier or vehicle can be particulate, so that the compositions are, for example, in tablet or powder form. The carrier(s) can be liquid, with the compositions being, for example, an oral syrup or injectable liquid. In addition, the carrier(s) can be gaseous, so as to provide an aerosol composition useful in, e.g., inhalatory administration.

[00212] When intended for oral administration, the composition is preferably in solid or liquid form, where semi-solid, semi-liquid, suspension and gel forms are included within the forms considered herein as either solid or liquid. As a solid composition for oral administration, the composition can be formulated into a powder, granule, compressed tablet, pill, capsule, chewing gum, wafer or the like form. Such a solid composition typically contains one or more inert diluents. In addition, one or more of the following can be present: binders such as carboxymethylcellulose, ethyl cellulose, microcrystalline cellulose, or gelatin; excipients such as starch, lactose or dextrans, disintegrating agents such as alginic acid, sodium.

## **Identification of Agonists and Antagonists for Molecules of the Invention**

### **Agonists and Antagonists—Assays and Molecules**

[00213] This invention provides a method of screening compounds to identify those which modulate the action of the polypeptide of the present invention. An example of such an assay comprises combining a mammalian fibroblast cell and the polypeptide(s) of the present invention, the compound to be screened and  $^3\text{[H]}$  thymidine under cell culture conditions where the fibroblast cell would normally proliferate. A control assay may be performed in the absence of the compound to be screened and compared to the amount of fibroblast proliferation in the presence of the compound to determine if the compound stimulates proliferation by determining the uptake of  $^3\text{[H]}$  thymidine in each case. The amount of fibroblast cell proliferation is measured by liquid scintillation chromatography which measures the incorporation of  $^3\text{[H]}$  thymidine. Both agonist and antagonist compounds may be identified by this procedure.

[00214] In another method, a mammalian cell or membrane preparation expressing a receptor for a polypeptide of the present invention, as described above, is incubated with a labeled polypeptide of the present invention in the presence of the compound. The ability of the compound to enhance or block this interaction could then be measured. Alternatively, the response of a known second messenger system following interaction of a compound to be screened and the Apo2 or IL-24 receptor is measured and the ability of the compound to bind to the receptor and elicit a second messenger response is measured to determine if the compound is a potential agonist or antagonist. Such second messenger systems include but are not limited to, cAMP guanylate cyclase, ion channels or phosphoinositide hydrolysis.

[00215] Examples of antagonist compounds include antibodies, or in some cases, oligonucleotides, which bind to the receptor for the polypeptide of the present invention but elicit no second messenger response or bind to the Apo2 or IL-24 polypeptide itself. Alternatively, a potential antagonist may be a mutant form of the polypeptide which binds to the receptors, however, no second messenger response is elicited and, therefore, the action of the polypeptide is effectively blocked.

[00216] Another antagonist compound to the Apo2 or IL-24 gene and gene product is an antisense construct prepared using antisense technology. Antisense technology can be used

to control gene expression through triple-helix formation or antisense DNA or RNA, both of which methods are based on binding of a polynucleotide to DNA or RNA. For example, the 5' coding portion of the polynucleotide sequence, which encodes for the mature polypeptides of the present invention, is used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription, for example, triple helix--see Lee et al., *Nucl. Acids Res.*, 6:3073 (1979); Cooney et al, *Science*, 241:456 (1988); and Dervan et al., *Science*, 251: 1360 (1991), thereby preventing transcription and the production of the polypeptides of the present invention. The antisense RNA oligonucleotide hybridizes to the mRNA in vivo and blocks translation of the mRNA molecule into the polypeptide, Antisense--Okano, *J. Neurochem.*, 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, Fla. (1988). The oligonucleotides described above can also be delivered to cells such that the antisense RNA or DNA may be expressed in vivo to inhibit production of the polypeptide.

[00217] Potential antagonist compounds also include small molecules which bind to and occupy the binding site of the receptors thereby making the receptor inaccessible to its polypeptide such that normal biological activity is prevented. Examples of small molecules include, but are not limited to, small peptides or peptide-like molecules. Antagonist compounds may be employed to inhibit the cell growth and proliferation effects of the polypeptides of the present invention on neoplastic cells and tissues, i.e. stimulation of angiogenesis of tumors, and, therefore, retard or prevent abnormal cellular growth and proliferation, for example, in tumor formation or growth.

[00218] The antagonists may also be employed to prevent hyper-vascular diseases, and prevent the proliferation of epithelial lens cells after extracapsular cataract surgery. Prevention of the mitogenic activity of the polypeptides of the present invention may also be desirous in cases such as restenosis after balloon angioplasty. The antagonists may also be employed to prevent the growth of scar tissue during wound healing.

### Chromosome Assays

[00219] In certain preferred embodiments relating to chromosomal mapping, the cDNA herein disclosed-is used to clone genomic nucleic acid of the Apo2 or IL-24 of the

invention. This can be accomplished using a variety of well known techniques and libraries, which generally are available commercially. The genomic DNA then is used for in situ chromosome mapping using well known techniques for this purpose. Therefore, the nucleic acid molecules of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. Moreover, there is a current need for identifying particular sites on the chromosome. Few chromosome marking reagents based on actual sequence data (repeat polymorphisms) are presently available for marking chromosomal location. The mapping of DNAs to chromosomes according to the present invention is an important first step in correlating those sequences with genes associated with disease.

[00220] Briefly, sequences can be mapped to chromosomes by preparing PCR primers from the cDNA. Computer analysis of the 3' untranslated region is used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the primer will yield an amplified fragment.

[00221] PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular DNA to a particular chromosome. Using the present invention with the same oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes or pools of large genomic clones in an analogous manner. Other mapping strategies that can similarly be used to map to its chromosome include in situ hybridization, prescreening with labeled flow-sorted chromosomes and preselection by hybridization to construct chromosome specific-cDNA libraries.

[00222] Fluorescence in situ hybridization (FISH) of a cDNA clone to a metaphase chromosomal spread can be used to provide a precise chromosomal location in one step. This technique can be used with cDNA as short as 50 or 60 bases. For a review of this technique, see Verma et al., Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York (1988).

[00223] Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. (Such

data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

[00224] Next, it is necessary to determine the differences in the cDNA or genomic sequence between affected and unaffected individuals. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease. With current resolution of physical mapping and genetic mapping techniques, a cDNA precisely localized to a chromosomal region associated with the disease could be one of between 50 and 500 potential causative genes. Note: this assumes 1 megabase mapping resolution and one gene per 20 kb.

[00225] Using methods described above, the Apo2 or IL-24 gene of the invention has been mapped by florescent in situ hybridization to human chromosome 8p21. The corresponding map position in the mouse includes several disease loci including ds (disorganization--developmental disruption) and wc (waved coat--homozygous lethality).

[00226] The present invention also provides methods for identifying agents, such as antibodies, which enhance or block the action of Apo2 or IL-24 molecules on cells, such as its interaction with Apo2 or IL-24-binding molecules such as receptor molecules. An agonist is a compound which increases the natural biological functions of Apo2 or IL-24 molecules or which functions in a manner similar to Apo2 or IL-24 molecules, while antagonists decrease or eliminate such functions.

[00227] For example, a cellular compartment, such as a membrane preparation, may be prepared from a cell that expresses a molecule that binds Apo2 or IL-24 molecules, such as a molecule of a signaling or regulatory pathway modulated by Apo2 or IL-24 molecules. The preparation is incubated with labeled Apo2 and IL-24 molecules in the absence or the presence of a candidate molecule which may be an Apo2 and IL-24 agonist or antagonist. The ability of the candidate molecule to bind the binding molecule or Apo2 or IL-24 molecules themselves is reflected in decreased binding of the labeled ligand. Molecules which bind gratuitously, that is, without inducing the effects of Apo2 or IL-24 molecules when bound to the Apo2 or IL-24 binding molecules, are most likely to be good

antagonists. Molecules that bind well and elicit effects that are the same as or closely related to Apo2 and/or IL-24 molecules may potentially prove to be good agonists.

[00228] Apo2 and IL-24-like effects of potential agonists and antagonists may be measured, for instance, by determining activity of a second messenger system following interaction of the candidate molecule with a cell or appropriate cell preparation, and comparing the effect with that of Apo2 and IL-24 molecules or molecules that elicit the same effects as Apo2 and IL-24. Second messenger systems that may be useful in this regard include but are not limited to AMP guanylate cyclase, ion channel or phosphoinositide hydrolysis second messenger systems.

[00229] Another example of an assay for Apo2 and/or IL-24 antagonists is a competitive assay that combines Apo2 or IL-24 molecules and a potential antagonist with membrane-bound Apo2 or IL-24 receptor molecules or recombinant Apo2 or IL-24 receptor molecules under appropriate conditions for a competitive inhibition assay. Apo2 or IL-24 molecules can be labeled, such as by radioactivity, such that the number of Apo2 or IL-24 molecules bound to a receptor molecule can be determined accurately to assess the effectiveness of the potential antagonist.

[00230] Potential antagonists include small organic molecules, peptides, polypeptides and antibodies that bind to a polypeptide of the invention, and thereby inhibit or extinguish its activity. Potential antagonists also may be small organic molecules, a peptide, a polypeptide such as a closely related protein or antibody that binds the same sites on a binding molecule, such as a receptor molecule, without inducing Apo2 or IL-24-induced activities, thereby preventing the action of Apo2 or IL-24 molecules by excluding Apo2 or IL-24 molecules from binding. Antagonists of the invention include fragments of the Apo2 and IL-24 molecules having the nucleic acid and amino acid sequences shown in Appendices A-F (SEQ ID NOs: 1-16).

[00231] Other potential antagonists include antisense molecules. Antisense technology can be used to control gene expression through antisense DNA or RNA or through triple-helix formation. Antisense techniques are discussed, for example, in Okano, *J. Neurochem.*, 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, Fla. (1988). Triple helix formation is discussed in, for instance, Lee et al., *Nucleic Acids Research*, 6:3073 (1979); Cooney et al., *Science*, 241:456 (1988); and

Dervan et al., *Science*, 251:1360 (1991). The methods are based on binding of a polynucleotide to a complementary DNA or RNA. For example, the 5' coding portion of a polynucleotide that encodes the mature polypeptide of the present invention may be used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription thereby preventing transcription and the production of Apo2 or IL-24 molecules. The antisense RNA oligonucleotide hybridizes to the mRNA *in vivo* and blocks translation of the mRNA molecule into an Apo2 or IL-24 polypeptide. The oligonucleotides described above can also be delivered to cells such that the antisense RNA or DNA may be expressed *in vivo* to inhibit production of Apo2 or IL-24 molecules.

#### **Therapeutic Uses of Apo2 and IL-24 Molecules, Apo2 and IL-24 Agonists and Apo2 and IL-24 Antagonists.**

[00232] Apo2 or IL-24 polynucleotides, polypeptides, agonists or antagonists of the invention may be used in developing treatments for any disorder mediated (directly or indirectly) by defective, or insufficient amounts of Apo2 or IL-24 molecules. Apo2 or IL-24 polypeptides, agonists or antagonists may be administered to a patient (e.g., mammal, preferably human) afflicted with such a disorder. Alternatively, a gene therapy approach may be applied to treat such disorders. Disclosure herein of Apo2 and IL-24 nucleotide sequences permits the detection of defective Apo2 and IL-24 genes, and the replacement thereof with normal Apo2 and IL-24-encoding genes. Defective genes may be detected in *in vitro* diagnostic assays, and by comparison of the Apo2 and IL-24 nucleotide sequences disclosed herein with that of an Apo2 and IL-24 gene derived from a patient suspected of harboring a defect in this gene.

[00233] The Apo2 and/or IL-24 polypeptides of the present invention may be employed to treat lymphoproliferative disease which results in lymphadenopathy, the Apo2 and IL-24 molecules may mediate apoptosis by stimulating clonal deletion of T-cells and may therefore, be employed to treat autoimmune disease to stimulate peripheral tolerance and cytotoxic T-cell mediated apoptosis. The Apo2 and/or IL-24 molecules may also be employed as a research tool in elucidating the biology of autoimmune disorders including systemic lupus erythematosus (SLE), Graves' disease, immunoproliferative disease



lymphadenopathy (IPL), angioimmunoproliferative lymphadenopathy (AIL), immunoblastic lymphadenopathy (IBL), rheumatoid arthritis, diabetes, and multiple sclerosis, allergies and to treat graft versus host disease.

[00234] The Apo2 and IL-24 polynucleotides, polypeptides and/or agonists or antagonists of the invention may also be used to treat, prevent, diagnose and/or prognose diseases which include, but are not limited to, autoimmune disorders, immunodeficiency disorders, and graft versus host disease.

[00235] The Apo2 and IL-24 polypeptides of the present invention may also be employed to inhibit neoplasia, such as tumor cell growth. The Apo2 and IL-24 polypeptides may be responsible for tumor destruction through apoptosis and cytotoxicity to certain cells.

[00236] Diseases associated with increased cell survival, or the inhibition of apoptosis, that may be treated, prevented, diagnosed and/or prognosed with the Apo2 or IL-24 polynucleotides, polypeptides and/or agonists or antagonists of the invention include, but are not limited to, cancers (such as follicular lymphomas, carcinomas with p53 mutations, and hormone-dependent tumors, including, but not limited to colon cancer, cardiac tumors, pancreatic cancer, melanoma, retinoblastoma, glioblastoma, lung cancer, intestinal cancer, testicular cancer, stomach cancer, neuroblastoma, myxoma, myoma, lymphoma, endothelioma, osteoblastoma, osteoclastoma, osteosarcoma, chondrosarcoma, adenoma, breast cancer, prostate cancer, Kaposi's sarcoma and ovarian cancer); autoimmune disorders (such as, multiple sclerosis, Sjogren's syndrome, Grave's disease, Hashimoto's thyroiditis, autoimmune diabetes, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis, autoimmune gastritis, autoimmune thrombocytopenic purpura, and rheumatoid arthritis) and viral infections (such as herpes viruses, pox viruses and adenoviruses), inflammation, graft vs. host disease (acute and/or chronic), acute graft rejection, and chronic graft rejection. In preferred embodiments, Apo2 or IL-24 polynucleotides, polypeptides, agonists, or antagonists of the invention are used to inhibit growth, progression, and/or metastasis of cancers, in particular those listed above or in the paragraph that follows.

[00237] Additional diseases or conditions associated with increased cell survival, that may be treated, prevented, diagnosed and/or prognosed with the Apo2 or IL-24 polynucleotides, polypeptides and/or agonists or antagonists of the invention include, but are not limited to,

progression, and/or metastases of malignancies and related disorders such as leukemia (including acute leukemias (e.g., acute lymphocytic leukemia, acute myelocytic leukemia (including myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia)) and chronic leukemias (e.g., chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia)), polycythemia vera, lymphomas (e.g., Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain diseases, and solid tumors including, but not limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyo sarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, melanoma, neuroblastoma and retinoblastoma.

[00238] Diseases associated with increased apoptosis, that may be treated, prevented, diagnosed and/or prognosed with the Apo2 or IL-24 polynucleotides, polypeptides and/or agonists or antagonists of the invention include, but are not limited to, AIDS; neurodegenerative disorders (such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, retinitis pigmentosa, cerebellar degeneration and brain tumor or prior associated disease); autoimmune disorders (such as, multiple sclerosis, Sjogren's syndrome, Grave's disease Hashimoto's thyroiditis, autoimmune diabetes, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus, immune-related glomerulonephritis, autoimmune gastritis, thrombocytopenic purpura, and rheumatoid arthritis) myelodysplastic syndromes (such as aplastic anemia), graft vs. host disease (acute and/or chronic), ischemic injury (such as that caused by myocardial infarction, stroke and

reperfusion injury), liver injury or disease (e.g., hepatitis related liver injury, cirrhosis, ischemia/reperfusion injury, cholestosis (bile duct injury) and liver cancer); toxin-induced liver disease (such as that caused by alcohol), septic shock, ulcerative colitis, cachexia and anorexia. In preferred embodiments, Apo2 or IL-24 polynucleotides, polypeptides, agonists, and/or antagonists are used to treat the diseases and disorders listed above.

[00239] Many of the pathologies associated with HIV are mediated by apoptosis, including HIV-induced nephropathy and HIV encephalitis. Thus, in additional preferred embodiments, Apo2 or IL-24 polynucleotides, polypeptides, agonists or antagonists of the invention are used to treat AIDS and pathologies associated with AIDS.

[00240] Another embodiment of the present invention is directed to the use of Apo2 or IL-24 polynucleotides, polypeptides, or antagonists to reduce Apo2 or IL-24-mediated death of T cells in HIV-infected patients. The role of T cell apoptosis in the development of AIDS has been the subject of a number of studies (see, for example, Meyaard et al., *Science*, 257:217-219 (1992); Groux et al., *J. Exp. Med.*, 175:331 (1992); and Oyaizu et al., in *Cell Activation and Apoptosis in HIV Infection*, Andrieu and Lu, Eds., Plenum Press, New York, pp. 101-114 (1995)). Fas-mediated apoptosis has been implicated in the loss of T cells in HIV individuals (Katsikis et al., *J. Exp. Med.* 181:2029-2036 (1995)). It is also likely that T cell apoptosis occurs through multiple mechanisms.

[00241] Activated human T cells are induced to undergo programmed cell death (apoptosis) upon triggering through the CD3/T cell receptor complex, a process termed activated-induced cell death (AICD). AICD of CD4 T cells isolated from HIV-infected asymptomatic individuals has been reported (Groux et al., *supra*). Thus, AICD may play a role in the depletion of CD4+ T cells and the progression to AIDS in HIV-infected individuals. Thus, the present invention provides a method of inhibiting Apo2 or IL-24-mediated T cell death in HIV patients, comprising administering Apo2 or IL-24 polynucleotides, polypeptides, or antagonists of the invention to the patients. In one embodiment, the patient is asymptomatic when treatment with Apo2 or IL-24 polynucleotides, polypeptides, or antagonists commences. If desired, prior to treatment, peripheral blood T cells may be extracted from an HIV patient, and tested for susceptibility to Apo2 or IL-24-mediated cell death by procedures known in the art. In one embodiment, a patient's blood or plasma is contacted with Apo2 or IL-24 antagonists (e.g., anti-Apo2 or IL-24 antibodies) of the invention ex

vivo. The Apo2 or IL-24 antagonists may be bound to a suitable chromatography matrix by procedures known in the art. The patient's blood or plasma flows through a chromatography column containing Apo2 or IL-24 antagonist bound to the matrix, before being returned to the patient. The immobilized Apo2 or IL-24 antagonist binds Apo2 or IL-24, thus removing Apo2 or IL-24 protein from the patient's blood.

[00242] In additional embodiments, an Apo2 or IL-24 polynucleotide, polypeptide, or antagonist of the invention is administered in combination with other inhibitors of T cell apoptosis. For example, as discussed above, Fas-mediated apoptosis also has been implicated in loss of T cells in HIV individuals (Katsikis et al., *J. Exp. Med.*, 181:2029-2036 (1995)). Thus, a patient susceptible to both Fas ligand mediated and Apo2 or IL-24-mediated T cell death may be treated with both an agent that blocks Apo2 or IL-24/Apo2 or IL-24 receptor interactions and an agent that blocks Fas-ligand/Fas interactions. Suitable agents for blocking binding of Fas-ligand to Fas include, but are not limited to, soluble Fas polypeptides; multimeric forms of soluble Fas polypeptides (e.g., dimers of sFas/Fc); anti-Fas antibodies that bind Fas without transducing the biological signal that results in apoptosis; anti-Fas-ligand antibodies that block binding of Fas-ligand to Fas; and muteins of Fas-ligand that bind Fas but do not transduce the biological signal that results in apoptosis. Preferably, the antibodies employed according to this method are monoclonal antibodies. Examples of suitable agents for blocking Fas-ligand/Fas interactions, including blocking anti-Fas monoclonal antibodies, are described in WO 95/10540, hereby incorporated by reference.

[00243] In another example, agents which block binding of Apo2 or IL-24 to an Apo2 or IL-24 receptor are administered with the Apo2 or IL-24 polynucleotides, polypeptides, or antagonists of the invention. Such agents include, but are not limited to, soluble Apo2 or IL-24 receptor polypeptides; multimeric forms of soluble Apo2 or IL-24 receptor polypeptides; and Apo2 or IL-24 receptor antibodies that bind the Apo2 or IL-24 receptor without transducing the biological signal that results in apoptosis, anti- Apo2 or IL-24 antibodies that block binding of Apo2 or IL-24 to one or more Apo2 or IL-24 receptors, and muteins of Apo2 or IL-24 that bind Apo2 or IL-24 receptors but do not transduce the biological signal that results in apoptosis.

[00244] Apo2 and/or IL-24 polypeptides of the invention may also be employed to regulate hematopoiesis and, in particular, erythropoiesis. Hematopoiesis is a multi-step cell proliferation and differentiation process which begins with a pool of multipotent stem cells. These cells can proliferate and differentiate into hematopoietic progenitors in reply to different stimuli. The Apo2 and/or IL-24 polypeptides of the invention, as well as agonists and antagonists thereof, may be used to either stimulate or inhibit development of hematopoietic cells and, in particular, erythropoietic precursor cells.

[00245] Additionally, molecules of the invention may be employed as an agent to boost immunoresponsiveness among individuals having a temporary immune deficiency. Conditions resulting in a temporary immune deficiency that may be ameliorated or treated by administering the Apo2 or IL-24 polypeptides or polynucleotides of the invention, or agonists thereof, include, but are not limited to, recovery from viral infections (e.g., influenza), conditions associated with malnutrition, recovery from infectious mononucleosis, or conditions associated with stress, recovery from measles, recovery from blood transfusion, recovery from surgery.

[00246] Apo2 or IL-24 polynucleotides or polypeptides of the invention, or agonists or antagonists thereof, may be used to diagnose, prognose, treat or prevent one or more of the following diseases or disorders, or conditions associated therewith: primary immunodeficiencies, immune-mediated thrombocytopenia, Kawasaki syndrome, bone marrow transplant (e.g., recent bone marrow transplant in adults or children), chronic B-cell lymphocytic leukemia, HIV infection (e.g., adult or pediatric HIV infection), chronic inflammatory demyelinating polyneuropathy, and post-transfusion purpura.

[00247] Additionally, Apo2 or IL-24 polynucleotides or polypeptides of the invention, or agonists or antagonists thereof, may be used to diagnose, prognose, treat or prevent one or more of the following diseases, disorders, or conditions associated therewith, Guillain-Barre syndrome, anemia (e.g., anemia associated with parvovirus B19, patients with stable multiple myeloma who are at high risk for infection (e.g., recurrent infection), autoimmune hemolytic anemia (e.g., warm-type autoimmune hemolytic anemia), thrombocytopenia (e.g., neonatal thrombocytopenia), and immune-mediated neutropenia), transplantation (e.g., cytomegalovirus (CMV)-negative recipients of CMV-positive organs), hypogammaglobulinemia (e.g., hypogammaglobulinemic neonates with risk factor for

infection or morbidity), epilepsy (e.g., intractable epilepsy), systemic vasculitic syndromes, myasthenia gravis (e.g., decompensation in myasthenia gravis), dermatomyositis, and polymyositis.

[00248] Autoimmune disorders and conditions associated with these disorders that may be treated, prevented, and/or diagnosed with the Apo2 or IL-24 polynucleotides, polypeptides, and/or antagonist of the invention (e.g., anti-Apo2 or IL-24 antibodies), include, but are not limited to, autoimmune hemolytic anemia, autoimmune neonatal thrombocytopenia, idiopathic thrombocytopenia purpura, autoimmunocytopenia, hemolytic anemia, antiphospholipid syndrome, dermatitis, allergic encephalomyelitis, myocarditis, relapsing polychondritis, rheumatic heart disease, glomerulonephritis (e.g., IgA nephropathy), Multiple Sclerosis, Neuritis, Uveitis Ophthalmia, Polyendocrinopathies, Purpura (e.g., Henloch-Schoenlein purpura), Reiter's disease, Stiff-Man Syndrome, Autoimmune Pulmonary Inflammation, Guillain-Barre Syndrome, insulin dependent diabetes mellitis, and autoimmune inflammatory eye disease.

[00249] Additional autoimmune disorders (that are highly probable) that may be treated, prevented, and/or diagnosed with the compositions of the invention include, but are not limited to, autoimmune thyroiditis, hypothyroidism (i.e., Hashimoto's thyroiditis) (often characterized, e.g., by cell-mediated and humoral thyroid cytotoxicity), systemic lupus erythematosus (often characterized, e.g., by circulating and locally generated immune complexes), Goodpasture's syndrome (often characterized, e.g., by anti-basement membrane antibodies), Pemphigus (often characterized, e.g., by epidermal acantholytic antibodies), receptor autoimmunities such as, for example, (a) Graves' disease (often characterized, e.g., by TSH receptor antibodies), (b) Myasthenia Gravis (often characterized, e.g., by acetylcholine receptor antibodies), and (c) insulin resistance (often characterized, e.g., by insulin receptor antibodies), autoimmune hemolytic anemia (often characterized, e.g., by phagocytosis of antibody-sensitized RBCs), autoimmune thrombocytopenic purpura (often characterized, e.g., by phagocytosis of antibody-sensitized platelets).

[00250] Additional autoimmune disorders (that are probable) that may be treated, prevented, and/or diagnosed with the compositions of the invention include, but are not limited to, rheumatoid arthritis (often characterized, e.g., by immune complexes in joints), scleroderma with anti-collagen antibodies (often characterized, e.g., by nucleolar and other

nuclear antibodies), mixed connective tissue disease (often characterized, e.g., by antibodies to extractable nuclear antigens (e.g., ribonucleoprotein)), polymyositis/dermatomyositis (often characterized, e.g., by nonhistone ANA), pernicious anemia (often characterized, e.g., by antiparietal cell, microsomes, and intrinsic factor antibodies), idiopathic Addison's disease (often characterized, e.g., by humoral and cell-mediated adrenal cytotoxicity, infertility (often characterized, e.g., by antispermatozoal antibodies), glomerulonephritis (often characterized, e.g., by glomerular basement membrane antibodies or immune complexes) such as primary glomerulonephritis and IgA nephropathy, bullous pemphigoid (often characterized, e.g., by IgG and complement in basement membrane), Sjogren's syndrome (often characterized, e.g., by multiple tissue antibodies, and/or a specific nonhistone ANA (SS-B)), diabetes mellitus (often characterized, e.g., by cell-mediated and humoral islet cell antibodies), and adrenergic drug resistance (including adrenergic drug resistance with asthma or cystic fibrosis) (often characterized, e.g., by beta-adrenergic receptor antibodies).

**[00251]** Additional autoimmune disorders (that are possible) that may be treated, prevented, and/or diagnosed with the compositions of the invention include, but are not limited to, chronic active hepatitis (often characterized, e.g. by smooth muscle antibodies), primary biliary cirrhosis (often characterized, e.g., by mitochondrial antibodies), other endocrine gland failure (often characterized, e.g., by specific tissue antibodies in some cases), vitiligo (often characterized, e.g., by melanocyte antibodies), vasculitis (often characterized, e.g., by Ig and complement in vessel walls and/or low serum complement), post-MI (often characterized, e.g., by myocardial antibodies), cardiomy syndrome (often characterized, e.g., by myocardial antibodies), urticaria (often characterized, e.g., by IgG and IgM antibodies to IgE), atopic dermatitis (often characterized, e.g., by IgG and IgM antibodies to IgE), asthma (often characterized, e.g., by IgG and IgM antibodies to IgE), inflammatory myopathies, and many other inflammatory, granulomatous, degenerative, and atrophic disorders.

**[00252]** In an additional embodiment, Apo2 or IL-24 polynucleotides or polypeptides, or antagonists thereof (e.g., anti-Apo2 or IL-24 antibodies) are used to treat or prevent systemic lupus erythematosus and/or diseases, disorders or conditions associated therewith. Lupus-associated diseases, disorders, or conditions that may be treated or prevented with

Apo2 or IL-24 polynucleotides or polypeptides, or antagonists of the invention, include, but are not limited to, hematologic disorders (e.g., hemolytic anemia, leukopenia, lymphopenia, and thrombocytopenia), immunologic disorders (e.g., anti-DNA antibodies, and anti-Sm antibodies), rashes, photosensitivity, oral ulcers, arthritis, fever, fatigue, weight loss, serositis (e.g., pleuritis (pleuricy)), renal disorders (e.g., nephritis), neurological disorders (e.g., seizures, peripheral neuropathy, CNS related disorders), gastrointestinal disorders, Raynaud phenomenon, and pericarditis.

[00253] Apo2 and/or IL-24 polypeptides, agonists or antagonists of the invention may be used to treat cardiovascular disorders, including peripheral artery disease, such as limb ischemia.

[00254] Cardiovascular disorders include cardiovascular abnormalities, such as arterio-arterial fistula, arteriovenous fistula, cerebral arteriovenous malformations, congenital heart defects, pulmonary atresia and Scimitar Syndrome. Congenital heart defects include aortic coarctation, cor triatriatum, coronary vessel anomalies, crisscross heart, dextrocardia, patent ductus arteriosus, Ebstein's anomaly, Eisenmenger complex, hypoplastic left heart syndrome, levocardia, tetralogy of fallot, transposition of great vessels, double outlet right ventricle, tricuspid atresia, persistent truncus arteriosus, and heart septal defects, such as aortopulmonary septal defect, endocardial cushion defects, Lutembacher's Syndrome, trilog of Fallot, and ventricular heart septal defects.

[00255] Cardiovascular disorders also include heart disease, such as arrhythmias, carcinoid heart disease, high cardiac output, low cardiac output, cardiac tamponade, endocarditis (including bacterial), heart aneurysm, cardiac arrest, congestive heart failure, congestive cardiomyopathy, paroxysmal dyspnea, cardiac edema, heart hypertrophy, congestive cardiomyopathy, left ventricular hypertrophy, right ventricular hypertrophy, post-infarction heart rupture, ventricular septal rupture, heart valve diseases, myocardial diseases, myocardial ischemia, pericardial effusion, pericarditis (including constrictive and tuberculous), pneumopericardium, postpericardiotomy syndrome, pulmonary heart disease, rheumatic heart disease, ventricular dysfunction, hyperemia, cardiovascular pregnancy complications, Scimitar Syndrome, cardiovascular syphilis, and cardiovascular tuberculosis.

[00256] Arrhythmias include sinus arrhythmia, atrial fibrillation, atrial flutter, bradycardia, extrasystole, Adams-Stokes Syndrome, bundle-branch block, sinoatrial block, long QT



syndrome, parasystole, Lown-Ganong-Levine Syndrome, Mahaim-type pre-excitation syndrome, Wolff-Parkinson-White syndrome, sick sinus syndrome, tachycardias, and ventricular fibrillation. Tachycardias include paroxysmal tachycardia, supraventricular tachycardia, accelerated idioventricular rhythm, atrioventricular nodal reentry tachycardia, ectopic atrial tachycardia, ectopic junctional tachycardia, sinoatrial nodal reentry tachycardia, sinus tachycardia, Torsades de Pointes, and ventricular tachycardia.

[00257] Heart valve disease include aortic valve insufficiency, aortic valve stenosis, heart murmurs, aortic valve prolapse, mitral valve prolapse, tricuspid valve prolapse, mitral valve insufficiency, mitral valve stenosis, pulmonary atresia, pulmonary valve insufficiency, pulmonary valve stenosis, tricuspid atresia, tricuspid valve insufficiency, and tricuspid valve stenosis.

[00258] Myocardial diseases include alcoholic cardiomyopathy, congestive cardiomyopathy, hypertrophic cardiomyopathy, aortic subvalvular stenosis, pulmonary subvalvular stenosis, restrictive cardiomyopathy, Chagas cardiomyopathy, endocardial fibroelastosis, endomyocardial fibrosis, Kearns Syndrome, myocardial reperfusion injury, and myocarditis.

[00259] Myocardial ischemias include coronary disease, such as angina pectoris, coronary aneurysm, coronary arteriosclerosis, coronary thrombosis, coronary vasospasm, myocardial infarction and myocardial stunning.

[00260] Cardiovascular diseases also include vascular diseases such as aneurysms, angiodyplasia, angiomatosis, bacillary angiomatosis, Hippel-Lindau Disease, Klippel-Trenaunay-Weber Syndrome, Sturge-Weber Syndrome, angioneurotic edema, aortic diseases, Takayasu's Arteritis, aortitis, Leriche's Syndrome, arterial occlusive diseases, arteritis, enarteritis, polyarteritis nodosa, cerebrovascular disorders, diabetic angiopathies, diabetic retinopathy, embolisms, thrombosis, erythromelalgia, hemorrhoids, hepatic veno-occlusive disease, hypertension, hypotension, ischemia, peripheral vascular diseases, phlebitis, pulmonary veno-occlusive disease, Raynaud's disease, CREST syndrome, retinal vein occlusion, Scimitar syndrome, superior vena cava syndrome, telangiectasia, atacia telangiectasia, hereditary hemorrhagic telangiectasia, varicocele, varicose veins, varicose ulcer, vasculitis, and venous insufficiency.

[00261] Aneurysms include dissecting aneurysms, false aneurysms, infected aneurysms, ruptured aneurysms, aortic aneurysms, cerebral aneurysms, coronary aneurysms, heart aneurysms, and iliac aneurysms.

[00262] Arterial occlusive diseases include arteriosclerosis, intermittent claudication, carotid stenosis, fibromuscular dysplasias, mesenteric vascular occlusion, Moyamoya disease, renal artery obstruction, retinal artery occlusion, and thromboangiitis obliterans.

[00263] Cerebrovascular disorders include carotid artery diseases, cerebral amyloid angiopathy, cerebral aneurysm, cerebral anoxia, cerebral arteriosclerosis, cerebral arteriovenous malformation, cerebral artery diseases, cerebral embolism and thrombosis, carotid artery thrombosis, sinus thrombosis, Wallenberg's syndrome, cerebral hemorrhage, epidural hematoma, subdural hematoma, subarachnoid hemorrhage, cerebral infarction, cerebral ischemia (including transient), subclavian steal syndrome, periventricular leukomalacia, vascular headache, cluster headache, migraine, and vertebrobasilar insufficiency.

[00264] Embolisms include air embolisms, amniotic fluid embolisms, cholesterol embolisms, blue toe syndrome, fat embolisms, pulmonary embolisms, and thromboembolisms. Thrombosis include coronary thrombosis, hepatic vein thrombosis, retinal vein occlusion, carotid artery thrombosis, sinus thrombosis, Wallenberg's syndrome, and thrombophlebitis.

[00265] Ischemia includes cerebral ischemia, ischemic colitis, compartment syndromes, anterior compartment syndrome, myocardial ischemia, reperfusion injuries, and peripheral limb ischemia. Vasculitis includes aortitis, arteritis, Behcet's Syndrome, Churg-Strauss Syndrome, mucocutaneous lymph node syndrome, thromboangiitis obliterans, hypersensitivity vasculitis, Schoenlein-Henoch purpura, allergic cutaneous vasculitis, and Wegener's granulomatosis.

[00266] The present invention further provides for treatment of diseases or disorders associated with neovascularization by administration of the Apo2 or IL-24 polynucleotides and/or polypeptides of the invention (including Apo2 or IL-24 agonists and/or antagonists). Malignant and metastatic conditions which can be treated with the polynucleotides and polypeptides of the invention include, but are not limited to those malignancies, solid

tumors, and cancers described herein and otherwise known in the art (for a review of such disorders, see Fishman et al., *Medicine*, 2d Ed., J. B. Lippincott Co., Philadelphia (1985)).

[00267] Additionally, ocular disorders associated with neovascularization which can be treated with the Apo2 or IL-24 polynucleotides and polypeptides of the present invention (including Apo2 or IL-24 agonists and Apo2 or IL-24 antagonists) include, but are not limited to: neovascular glaucoma, diabetic retinopathy, retinoblastoma, retrolental fibroplasia, uveitis, retinopathy of prematurity, macular degeneration, corneal graft neovascularization, as well as other eye inflammatory diseases, ocular tumors and diseases associated with choroidal or iris neovascularization. See, e.g., reviews by Waltman et al., *Am. J. Ophthalmol.*, 85:704-710 (1978) and Gartner et al., *Surv. Ophthalmol.*, 22:291-312 (1978).

[00268] Additionally, disorders which can be treated with the Apo2 or IL-24 polynucleotides and polypeptides of the present invention (including Apo2 or IL-24 agonists and Apo2 or IL-24 antagonists) include, but are not limited to, hemangioma, arthritis, psoriasis, angiofibroma, atherosclerotic plaques, delayed wound healing, granulations, hemophilic joints, hypertrophic scars, nonunion fractures, Osler-Weber syndrome, pyogenic granuloma, scleroderma, trachoma, and vascular adhesions.

[00269] Polynucleotides and/or polypeptides of the invention, and/or agonists and/or antagonists thereof, are useful in the diagnosis and treatment or prevention of a wide range of diseases and/or conditions. Such diseases and conditions include, but are not limited to, cancer (e.g., immune cell related cancers, breast cancer, prostate cancer, ovarian cancer, follicular lymphoma, cancer associated with mutation or alteration of p53, brain tumor, bladder cancer, uterocervical cancer, colon cancer, colorectal cancer, non-small cell carcinoma of the lung, small cell carcinoma of the lung, stomach cancer, etc.), lymphoproliferative disorders (e.g., lymphadenopathy), microbial (e.g., viral, bacterial, etc.) infection (e.g., HIV-1 infection, HIV-2 infection, herpesvirus infection (including, but not limited to, HSV-1, HSV-2, CMV, VZV, HHV-6, HHV-7, EBV), adenovirus infection, poxvirus infection, human papilloma virus infection, hepatitis infection (e.g., HAV, HBV, HCV, etc.), *Helicobacter pylori* infection, invasive *Staphylococcus*, etc.), parasitic infection, nephritis, bone disease (e.g., osteoporosis), atherosclerosis, pain, cardiovascular disorders (e.g., neovascularization, hypovascularization or reduced circulation (e.g., ischemic disease (e.g., myocardial infarction, stroke, etc.)), AIDS, allergy, inflammation, neurodegenerative

disease (e.g., Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, pigmentary retinitis, cerebellar degeneration, etc.), graft rejection (acute and chronic), graft vs. host disease, diseases due to osteomyelodysplasia (e.g., aplastic anemia, etc.), joint tissue destruction in rheumatism, liver disease (e.g., acute and chronic hepatitis, liver injury, and cirrhosis), autoimmune disease (e.g., multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, immune complex glomerulonephritis, autoimmune diabetes, autoimmune thrombocytopenic purpura, Grave's disease, Hashimoto's thyroiditis, etc.), cardiomyopathy (e.g., dilated cardiomyopathy), diabetes, diabetic complications (e.g., diabetic nephropathy, diabetic neuropathy, diabetic retinopathy), influenza, asthma, psoriasis, glomerulonephritis, septic shock, and ulcerative colitis.

**[00270]** Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof are useful in promoting angiogenesis, wound healing (e.g., wounds, burns, and bone fractures).

**[00271]** Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof are also useful as an adjuvant to enhance immune responsiveness to specific antigen and/or anti-viral immune responses.

**[00272]** More generally, polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof are useful in regulating (i.e., elevating or reducing) immune response. For example, polynucleotides and/or polypeptides of the invention may be useful in preparation or recovery from surgery, trauma, radiation therapy, chemotherapy, and transplantation, or may be used to boost immune response and/or recovery in the elderly and immunocompromised individuals. Alternatively, polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof are useful as immunosuppressive agents, for example in the treatment or prevention of autoimmune disorders. In specific embodiments, polynucleotides and/or polypeptides of the invention are used to treat or prevent chronic inflammatory, allergic or autoimmune conditions, such as those described herein or are otherwise known in the art/

**[00273]** The uses of the Apo2 or IL-24 polypeptides, include but are not limited to, the treatment or prevention of viral hepatitis, Herpes viral infections, allergic reactions, adult respiratory distress syndrome, neoplasia, anaphylaxis, allergic asthma, allergen rhinitis, drug allergies (e.g., to penicillin, cephalosporins), primary central nervous system

lymphoma (PCNSL), glioblastoma, chronic lymphocytic leukemia (CLL), lymphadenopathy, autoimmune disease, graft versus host disease, rheumatoid arthritis, osteoarthritis, Graves' disease, acute lymphoblastic leukemia (ALL), lymphomas (Hodgkin's disease and non-Hodgkin's lymphoma (NHL)), ophthalmopathy, uveoretinitis, the autoimmune phase of Type 1 diabetes, myasthenia gravis, glomerulonephritis, autoimmune hepatological disorder, autoimmune inflammatory bowel disease, and Crohn's disease. In addition, the APO2 OR IL-24 polypeptide of the present invention may be employed to inhibit neoplasia, such as tumor cell growth. The combination of Apo2 or IL-24 protein with immunotherapeutic agent such as IL-2 or IL-12 may result in synergistic or additive effects that would be useful for the treatment of established cancers.

#### Antibodies

[00274] Apo2 or IL-24-protein specific antibodies for use in the present invention can be raised against the intact Apo2 or IL-24 protein or an antigenic polypeptide fragment thereof, which may be presented together with a carrier protein, such as an albumin, to an animal system (such as rabbit or mouse) or, if it is long enough (at least about 25 amino acids), without a carrier.

[00275] The antibodies of the present invention may be prepared by any of a variety of methods. For example, cells expressing the Apo2 or IL-24 protein or an antigenic fragment thereof can be administered to an animal in order to induce the production of sera containing polyclonal antibodies. In a preferred method, a preparation of Apo2 or IL-24 protein is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

[00276] In the most preferred method, the antibodies of the present invention are monoclonal antibodies, or Apo2 or IL-24 protein binding fragments thereof. Such monoclonal antibodies can be prepared using hybridoma technology, for example, Kohler et al., *Nature*, 256:495 (1975); Kohler et al., *Eur. J. Immunol.*, 6:511 (1976); Kohler et. al., *Eur. J. Immunol.*, 6:292 (1976); Hammerling et al., in: *Monoclonal Antibodies and T-Cell Hybridomas*, Elsevier, N.Y., (1981) pp. 563-681). In general, such procedures involve immunizing an animal (preferably a mouse) with an Apo2 or IL-24 protein antigen or, more

preferably, with an Apo2 or IL-24 protein-expressing cell. Suitable cells can be recognized by their capacity to bind anti-Apo2 or IL-24 protein antibody. Such cells may be cultured in any suitable tissue culture medium; however, it is preferable to culture cells in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56°C), and supplemented with about 10 grams/liter of nonessential amino acids, about 1,000 U/ml of penicillin, and about 100 µg/ml of streptomycin. The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP20), available from the American Type Culture Collection, Manassas, Va. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al., *Gastroenterology*, 80:225-232 (1981).

#### **Apo2 and IL-24 protein antigen**

[00277] Alternatively, additional antibodies capable of binding to the Apo2 or IL-24 protein antigen may be produced in a two-step procedure through the use of anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and that, therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, Apo2 or IL-24-protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the Apo2 or IL-24 protein-specific antibody can be blocked by the Apo2 or IL-24 protein antigen. Such antibodies comprise anti-idiotypic antibodies to the Apo2 or IL-24 protein-specific antibody and can be used to immunize an animal to induce formation of further Apo2 or IL-24 protein-specific antibodies.

[00278] It will be appreciated that Fab and F(ab')<sub>2</sub> and other fragments of the antibodies of the present invention may be used according to the methods disclosed herein. Such fragments are typically produced by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')<sub>2</sub> fragments). Alternatively, Apo2 or IL-24 protein-binding fragments can be produced through the application of recombinant

DNA technology or through synthetic chemistry. For in vivo use of anti- Apo2 or IL-24 in humans, it may be preferable to use "humanized" chimeric monoclonal antibodies. Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric antibodies are known in the art. See, for review, Morrison, *Science*, 229:1202 (1985); Oi et al., *BioTechniques*, 4:214 (1986); Cabilly et al., U.S. Pat. No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et al., WO 8702671; Boulianne et al., *Nature*, 312:643 (1984); Neuberger et al., *Nature*, 314:268 (1985).

### Diagnosis

[00279] This invention is also related to the use of the genes of the present invention as part of a diagnostic assay for detecting diseases or susceptibility to diseases related to the presence of mutations in the nucleic acid sequences encoding the polypeptide of the present invention. Individuals carrying mutations in a gene of the present invention may be detected at the DNA level by a variety of techniques. Nucleic acids for diagnosis may be obtained from a patient's cells, such as from blood, urine, saliva, tissue biopsy and autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR, for example, Saiki et al., *Nature*, 324: 163-166 (1986), prior to analysis. RNA or cDNA may also be used for the same purpose. As an example, PCR primers complementary to the nucleic acid encoding a polypeptide of the present invention can be used to identify and analyze mutations. For example, deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to radiolabeled RNA or alternatively, radiolabeled antisense DNA sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase A digestion or by differences in melting temperatures.

[00280] Genetic testing based on DNA sequence differences may be achieved by detection of alteration in electrophoretic mobility of DNA fragments in gels with or without denaturing agents. Small sequence deletions and insertions can be visualized by high resolution gel electrophoresis. DNA fragments of different sequences may be distinguished

on denaturing formamide gradient gels in which the mobilities of different DNA fragments are retarded in the gel at different positions according to their specific melting or partial melting temperatures, for example, Myers et al., *Science*, 230:1242 (1985).

[00281] Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method as shown in Cotton et al., PNAS, USA, 85:4397-4401 (1985). Thus, the detection of a specific DNA sequence may be achieved by methods such as hybridization, RNase protection, chemical cleavage, direct DNA sequencing or the use of restriction enzymes, (e.g., Restriction Fragment Length Polymorphisms (RFLP)) and Southern blotting of genomic DNA. In addition to more conventional gel-electrophoresis and DNA sequencing, mutations can also be detected by in situ analysis.

[00282] The present invention also relates to a diagnostic assay for detecting altered levels of Apo2 or IL-24 proteins in various tissues since an over-expression of the proteins compared to normal control tissue samples may detect the presence of abnormal cellular proliferation, for example, a tumor. Assays used to detect levels of protein in a sample derived from a host are well-known to those of skill in the art and include radioimmunoassays, competitive-binding assays, Western Blot analysis, ELISA assays and "sandwich" assay. An ELISA assay, for example, Coligan, et al., *Current Protocols in Immunology*, 1(2), Chapter 6, (1991), initially contains preparing an antibody specific to an antigen to the polypeptides of the present invention, preferably a monoclonal antibody.

[00283] In addition a reporter antibody is prepared against the monoclonal antibody. To the reporter antibody is attached a detectable reagent such as radioactivity, fluorescence or, in this example, a horseradish peroxidase enzyme. A sample is removed from a host and incubated on a solid support, e.g. a polystyrene dish, that binds the proteins in the sample. Any free protein binding sites on the dish are then covered by incubating with a non-specific protein like bovine serum albumen. Next, the monoclonal antibody is incubated in the dish during which time the monoclonal antibodies attach to any polypeptides of the present invention attached to the polystyrene dish. All unbound monoclonal antibody is washed out with buffer. The reporter antibody linked to horseradish peroxidase is now placed in the dish resulting in binding of the reporter antibody to any monoclonal antibody bound to the protein of interest. Unattached reporter antibody is then washed out.



Peroxidase substrates are then added to the dish and the amount of color developed in a given time period is a measurement of the amount of a polypeptide of the present invention present in a given volume of patient sample when compared against a standard curve.

[00284] A competition assay may be employed wherein antibodies specific to a polypeptide of the present invention are attached to a solid support and labeled Apo2 or IL-24 and a sample derived from the host are passed over the solid support and the amount of label detected, for example by liquid scintillation chromatography, can be correlated to a quantity of a polypeptide of the present invention in the sample. A “sandwich” assay is similar to an ELISA assay. In a “sandwich” assay a polypeptide of the present invention is passed over a solid support and binds to antibody attached to a solid support. A second antibody is then bound to the polypeptide of interest. A third antibody which is labeled and specific to the second antibody is then passed over the solid support and binds to the second antibody and an amount can then be quantified.

[00285] By “assaying the expression level of the gene encoding the Apo2 or IL-24 protein” is intended qualitatively or quantitatively measuring or estimating the level of the Apo2 or IL-24 protein or the level of the mRNA encoding the Apo2 or IL-24 protein in a first biological sample either directly, for example, by determining or estimating absolute protein level or mRNA level, or relatively, by comparing to the Apo2 or IL-24 protein level or mRNA level in a second biological sample. Preferably, the Apo2 or IL-24 protein level or mRNA level in the first biological sample is measured or estimated and compared to a standard Apo2 or IL-24 protein level or mRNA level, the standard being taken from a second biological sample obtained from an individual not having the disorder or being determined by averaging levels from a population of individuals not having a disorder related to Apo2 or IL-24 expression. As will be appreciated in the art, once a standard Apo2 or IL-24 protein level or mRNA level is known, it can be used repeatedly as a standard for comparison.

[00286] By “biological sample” is intended any biological sample obtained from a subject, body fluid, cell line, tissue culture, or other source which contains Apo2 or IL-24 protein or mRNA. As indicated, biological samples include body fluids (such as sera, plasma, urine, synovial fluid and spinal fluid) which contain free Apo2 or IL-24 protein, ovarian or renal system tissue, and other tissue sources found to express complete or mature Apo2 or IL-24

polypeptide or an Apo2 or IL-24 receptor. Methods for obtaining tissue biopsies and body fluids from mammals are well known in the art. Where the biological sample is to include mRNA, a tissue biopsy is the preferred source.

[00287] Total cellular RNA can be isolated from a biological sample using any suitable technique such as the single-step guanidinium-thiocyanate-phenol-chloroform method described in Chomczynski and Sacchi, *Anal. Biochem.*, 162:156-159 (1987). Levels of mRNA encoding the Apo2 or IL-24 protein are then assayed using any appropriate method. These include Northern blot analysis, S1 nuclease mapping, the polymerase chain reaction (PCR), reverse transcription in combination with the polymerase chain reaction (RT-PCR), and reverse transcription in combination with the ligase chain reaction (RT-LCR).

[00288] Assaying Apo2 or IL-24 protein levels in a biological sample can occur using antibody-based techniques. For example, Apo2 IL-24 protein expression in tissues can be studied with classical immunohistological methods, for example, Jalkanen, M., et al., *J. Cell. Biol.*, 101:976-985 (1985); Jalkanen, M., et al., *J. Cell. Biol.*, 105:3087-3096 (1987). Other antibody-based methods useful for detecting Apo2 or IL-24 protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase, radioisotopes, and fluorescent labels, such as fluorescein and rhodamine, and biotin.

[00289] In addition to assaying Apo2 or IL-24 protein levels in a biological sample obtained from an individual, Apo2 or IL-24 protein can also be detected in vivo by imaging. Antibody labels or markers for in vivo imaging of Apo2 or IL-24 protein include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to a subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody by labeling of nutrients for the relevant hybridoma.

[00290] An Apo2 or IL-24 protein-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope, a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced, for example, parenterally, subcutaneously or intraperitoneally, into the subject to be examined

for immune system disorder. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain Apo2 or IL-24 protein. In vivo tumor imaging is described in S. W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments", Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S. W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).

### **Formulations**

- [00291] The Apo2 or IL-24 polypeptide composition will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual subject, the site of delivery of the Apo2 or IL-24 polypeptide composition, the method of administration, the scheduling of administration, and other factors known to practitioners. The "effective amount" of Apo2 or IL-24 polypeptide for purposes herein is thus determined by such considerations.
- [00292] The polypeptides, agonists and antagonists of the present invention may be employed in combination with a suitable pharmaceutical carrier to comprise a pharmaceutical composition for parenteral administration. Such compositions comprise a therapeutically effective amount of the polypeptide, agonist or antagonist and a pharmaceutically acceptable carrier or excipient. Such a carrier includes but is not limited to saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The formulation should suit the mode of administration.
- [00293] The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the polypeptides, agonists and antagonists of the present invention may be employed in conjunction with other therapeutic compounds.
- [00294] The pharmaceutical compositions may be administered in a convenient manner such as by the oral, topical, intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal

or intradermal routes. The pharmaceutical compositions are administered in an amount which is effective for treating and/or prophylaxis of the specific indication. In general, they are administered in an amount of at least about 10 .mu.g/kg body weight and in most cases they will be administered in an amount not in excess of about 8 mg/Kg body weight per day.

[00295] The polypeptide of the invention and agonist and antagonist compounds which are polypeptides, may also be employed in accordance with the present invention by expression of such polypeptide in vivo, which is often referred to as "gene therapy." Thus, for example, cells may be engineered with a polynucleotide (DNA or RNA) encoding for the polypeptide ex vivo, the engineered cells are then provided to a patient to be treated with the polypeptide. Such methods are well-known in the art. For example, cells may be engineered by procedures known in the art by use of a retroviral particle containing RNA encoding for the polypeptide of the present invention.

[00296] Similarly, cells may be engineered in vivo for expression of the polypeptide in vivo, for example, by procedures known in the art. As known in the art, a producer cell for producing a retroviral particle containing RNA encoding the polypeptide of the present invention may be administered to a patient for engineering cells in vivo and expression of the polypeptide in vivo. These and other methods for administering a polypeptide of the present invention by such methods should be apparent to those skilled in the art from the teachings of the present invention. For example, the expression vehicle for engineering cells may be other than a retroviral particle, for example, an adenovirus, which may be used to engineer cells in vivo after combination with a suitable delivery vehicle.

[00297] Retroviruses from which the retroviral plasmid vectors hereinabove mentioned may be derived include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, retroviruses such as Rous Sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, gibbon ape leukemia virus, human immunodeficiency virus, adenovirus, .Myeloproliferative Sarcoma Virus, and mammary tumor virus. In one embodiment, the retroviral plasmid vector is derived from Moloney Murinen Leukemia Virus.

[00298] The vector includes one or-more promoters. Suitable promoters which may be employed include, but are not limited to, the retroviral LTR; the SV40 promoter; and the human cytomegalovirus (CMV) promoter described in Miller, et al., *Biotechniques*, Vol. 7,

No. 9, 980-990 (1989), or any other promoter, for example, cellular promoters such as eukaryotic cellular promoters including, but not limited to, the histone, pol III, and  $\beta$ -actin promoters. Other viral promoters which may be employed include, but are not limited to, adenovirus promoters, thymidine kinase (TK) promoters, and B19 parvovirus promoters. The selection of a suitable promoter will be apparent to those skilled in the art from the teachings contained herein.

[00299] The nucleic acid sequence encoding the polypeptide of the present invention is under the control of a suitable promoter. Suitable promoters which may be employed include, but are not limited to, adenoviral promoters, such as the adenoviral major late promoter; or heterologous promoters, such as the cytomegalovirus (CMV) promoter; the respiratory syncytial virus (RSV) promoter; inducible promoters, such as the MMT promoter, the metallothionein promoter; heat shock promoters; the albumin promoter; the ApoA1 promoter; human globin promoters; viral thymidine kinase promoters, such as the Herpes Simplex thymidine kinase promoter; retroviral LTRs (including the modified retroviral LTRs hereinabove described); the  $\beta$ -actin promoter; and human growth hormone promoters. The promoter also may be the native promoter which controls the gene encoding the polypeptide.

[00300] The retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected include, but are not limited to, the PE501, PA317, -2, -AM, PA12, T19-14X, VT-19-17-H2, CRE, CRIP, GP+E-86, GP+envAm12, and DAN cell lines as described in Miller, *Human Gene Therapy*, 1:5-14 (1990). The vector may transduce the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and  $\text{CaPO}_4$  precipitation.

[00301] In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host.

[00302] The producer cell line generates infectious retroviral vector particles which include the nucleic acid sequence(s) encoding the polypeptides. Such retroviral vector particles then may be employed, to transduce eukaryotic cells, either in vitro or in vivo. The transduced eukaryotic cells will express the nucleic acid sequence(s) encoding the polypeptide. Eukaryotic cells which may be transduced include, but are not limited to, embryonic stem

cells, embryonic carcinoma cells, as well as hematopoietic stem cells, hepatocytes, fibroblasts, myoblasts, keratinocytes, endothelial cells, and bronchial epithelial cells.

### **Therapeutic uses of the Molecules of the Invention**

[00303] The molecules of the invention are useful for treating cancer, an immunity disease, such as an autoimmune disease or an inflammatory disease, an ischemic disease or an infectious disease in an animal.

### **Treatment of Cancer**

[00304] The molecules of the invention are useful for inhibiting the multiplication of a tumor cell or cancer cell, or for treating cancer in an animal. The molecules of the invention can be used accordingly in a variety of settings for the treatment of animal cancers. Other particular types of cancers that can be treated with molecules of the Invention include, but are not limited to, those disclosed below:

Solid tumors, including but not limited to:

fibrosarcoma  
myxosarcoma  
liposarcoma  
chondrosarcoma  
osteogenic sarcoma  
chordoma  
angiosarcoma  
endotheliosarcoma  
lymphangiosarcoma  
lymphangioendotheliosarcoma  
synovioma  
mesothelioma  
Ewing's tumor  
leiomyosarcoma  
rhabdomyosarcoma  
colon cancer  
colorectal cancer  
kidney cancer  
pancreatic cancer  
bone cancer  
breast cancer  
ovarian cancer  
prostate cancer  
esophageal cancer

stomach cancer  
oral cancer  
nasal cancer  
throat cancer  
squamous cell carcinoma  
basal cell carcinoma  
adenocarcinoma  
sweat gland carcinoma  
sebaceous gland carcinoma  
papillary carcinoma  
papillary adenocarcinomas  
cystadenocarcinoma  
medullary carcinoma  
bronchogenic carcinoma  
renal cell carcinoma  
hepatoma  
bile duct carcinoma  
choriocarcinoma  
seminoma  
embryonal carcinoma  
Wilmsy tumor  
cervical cancer  
uterine cancer  
testicular cancer  
small cell lung carcinoma  
bladder carcinoma  
lung cancer  
epithelial carcinoma  
glioma  
glioblastoma multiforme  
astrocytoma  
medulloblastoma  
craniopharyngioma  
ependymoma  
pinealoma  
hemangioblastoma  
acoustic neuroma  
oligodendroglioma  
meningioma  
skin cancer  
melanoma  
neuroblastoma  
retinoblastoma  
blood-borne cancers, including but not limited to:  
acute lymphoblastic leukemia "ALL"  
acute lymphoblastic B-cell leukemia

acute lymphoblastic T-cell leukemia  
acute myeloblastic leukemia "AML"  
acute promyelocytic leukemia "APL"  
acute monoblastic leukemia  
acute erythroleukemic leukemia  
acute megakaryoblastic leukemia  
acute myelomonocytic leukemia  
acute nonlymphocytic leukemia  
acute undifferentiated leukemia  
chronic myelocytic leukemia "CML"  
chronic lymphocytic leukemia "CLL"  
hairy cell leukemia  
multiple myeloma  
acute and chronic leukemias:  
  lymphoblastic  
  myelogenous  
  lymphocytic  
  myelocytic leukemias

Lymphomas, including but not limited to:  
Hodgkin's disease  
non-Hodgkin's Lymphoma  
Multiple myeloma  
Waldenstrom's macroglobulinemia  
Heavy chain disease  
Polycythemia Vera

### **Treatment of Immunity Diseases**

[00305]       Molecules of the invention are useful for killing or inhibiting the replication of a cell that produces an autoimmune disease or an inflammatory disease or for treating an autoimmune disease or an inflammatory disease. Molecules of the invention can be used accordingly in a variety of settings for the treatment of an autoimmune disease or an inflammatory disease in an animal.

[00306]       Particular types of autoimmune diseases that can be treated with the molecules of the invention include, but are not limited to, Th2-lymphocyte related disorders (e.g., atopic dermatitis, atopic asthma, rhinoconjunctivitis, allergic rhinitis, Omenn's syndrome, systemic sclerosis, and graft versus host disease); Th-1 lymphocyte-related disorders (e.g., rheumatoid arthritis, multiple sclerosis, psoriasis, Sjorgren's syndrome, Hashimoto's thyroiditis, Grave's disease, primary biliary cirrhosis, Wegener's granulomatosis, and tuberculosis); activated B



lymphocyte-related disorders (e.g., systemic lupus erythematosus, Goodpasture's syndrome, rheumatoid arthritis, and type I diabetes); and those disclosed below:

Active Chronic Hepatitis  
Addison's Disease  
Allergic Alveolitis  
Allergic Reaction  
Allergic Rhinitis  
Alport's Syndrome  
Anaphlaxis  
Ankylosing Spondylitis  
Anti-phospholipid Syndrome  
Arthritis  
Ascariasis  
Aspergillosis  
Atopic Allergy  
Atopic Dermatitis  
Atopic Rhinitis  
Behcet's Disease  
Bird-Fancier 's Lung  
Bronchial Asthma  
Caplan's Syndrome  
Cardiomyopathy  
Celiac Disease  
Chagas' Disease  
Chronic Glomerulonephritis  
Cogan's Syndrome  
Cold Agglutinin Disease  
Congenital Rubella Infection  
CREST Syndrome  
Crohn's Disease  
Cryoglobulinemia  
Gushing's Syndrome  
Dermatomyositis  
Discoid Lupus  
Dressler's Syndrome  
Eaton-Lambert Syndrome  
Echovirus Infection  
Encephalomyelitis  
Endocrine ophthalmopathy  
Epstein-Barr Virus Infection  
Equine Heaves  
Erythematosis  
Evan's Syndrome  
Felty's Syndrome  
Fibromyalgia

Fuch's Cyclitis  
Gastric Atrophy  
Gastrointestinal Allergy  
Giant Cell Arteritis  
Glomerulonephritis  
Goodpasture's Syndrome  
Graft v. Host Disease  
Graves' Disease  
Guillain-Barre Disease  
Hashimoto's Thyroiditis  
Hemolytic Anemia  
Henoch-Schonlein Pwपुरa  
Idiopathic Adrenal Atrophy  
Idiopathic Pulmonary Fibrilis  
IgA Nephropathy  
Inflammatory Bowel Diseases  
Insulin-dependent Diabetes Mellitus  
Juvenile Arthritis  
Juvenile Diabetes Mellitus (Type I)  
Lambert-Eaton Syndrome  
Laminitis  
Lichen Planus  
Lupoid Hepatitis  
Lupus  
Lymphopenia  
Meniere's Disease  
Mixed Connective Tissue Disease  
Multiple Sclerosis  
Myasthenia Gravis  
Pernicious Anemia  
Polyglandular Syndromes  
Presenile Dementia  
Primary Agammaglobulinemia  
Primary Biliary Cirrhosis  
Psoriasis  
Psoriatic Arthritis  
Raynauds Phenomenon  
Recurrent Abortion  
Reiter' s Syndrome  
Rheumatic Fever  
Rheumatoid Arthritis  
Sampter's Syndrome  
Schistosomiasis  
Schmidt's Syndrome  
Scleroderma  
Shulman's Syndrome

Sjorgen's Syndrome  
Stiff-Man Syndrome  
Sympathetic Ophthalmia  
Systemic Lupus Erythematosus  
Takayasu's Arteritis  
Temporal Arteritis  
Thyroiditis  
Thrombocytopenia  
Thyrotoxicosis  
Toxic Epidermal Necrolysis  
Type B Insulin Resistance  
Type I Diabetes Mellitus  
Ulcerative Colitis  
Uveitis  
Vitiligo  
Waldenstrom's Macroglobulemia  
Wegener's Granulomatosis

#### **Treatment of Infectious Diseases**

[00307] Molecules of the invention are useful for killing or inhibiting the multiplication of a cell that produces an infectious disease or for treating an infectious disease. The molecules of the invention can be used accordingly in a variety of settings for the treatment of an infectious disease in an animal.

[00308] Particular types of infectious diseases that can be treated with molecules of the invention include, but are not limited to, those disclosed below:

##### **Bacterial Diseases:**

Diphtheria  
Pertussis  
Occult Bacteremia  
Urinary Tract Infection  
Gastroenteritis  
Cellulitis  
Epiglottitis  
Tracheitis  
Adenoid Hypertrophy  
Retropharyngeal Abscess  
Impetigo  
Ecthyma  
Pneumonia  
Endocarditis  
Septic Arthritis  
Pneumococcal

Peritonitis  
Bacteremia  
Meningitis  
Acute Purulent Meningitis  
Urethritis  
Cervicitis  
Proctitis  
Pharyngitis  
Salpingitis  
Epididynitis  
Gonorrhea  
Syphilis  
Listeriosis  
Anthrax  
Nocardiosis  
Salmonella  
Typhoid Fever  
Dysentery  
Conjunctivitis  
Sinusitis  
Brucellosis  
Tularemia  
Cholera  
Bubonic Plague  
Tetanus  
Necrotizing Enteritis  
Actinomycosis  
Mixed Anaerobic Infections  
Syphilis  
Relapsing Fever  
Leptospirosis  
Lyme Disease  
Rat Bite Fever  
Tuberculosis  
Lymphadenitis  
Leprosy  
Chlamydia  
Chlamydial Pneumonia  
Trachoma  
Inclusion Conjunctivitis  
Systemic Fungal Diseases:  
Histoplasmosis  
Coccidioidomycosis  
Blastomycosis  
Sporotrichosis  
Cryptococcosis

Systemic Candidiasis  
Aspergillosis  
Mucomycosis  
Mycetoma  
Chromomycosis  
Rickettsial Diseases:  
Typhus  
Rocky Mountain Spotted Fever  
Ehrlichiosis  
Eastern Tick-Borne Rickettsioses  
Rickettsialpox  
Q Fever  
Bartonellosis  
Parasitic Diseases:  
Malaria  
Babesiosis  
African Sleeping Sickness  
Chagas' Disease  
Leishmaniasis  
Dum-Dum Fever  
Toxoplasmosis  
Meningoencephalitis  
Keratitis  
Entamebiasis  
Giardiasis  
Cryptosporidiasis  
Isosporiasis  
Cyclosporiasis  
Microsporidiosis  
Ascariasis  
Whipworm Infection  
Hookworm Infection  
Threadworm Infection  
Ocular Larva Migrans  
Trichinosis  
Guinea Worm Disease  
Lymphatic Filariasis  
Loiasis  
River Blindness  
Canine Heartworm Infection  
Schistosomiasis  
Swimmer's Itch  
Oriental Lung Fluke  
Oriental Liver Fluke  
Fascioliasis  
Fasciolopsiasis

Opisthorchiasis  
Tapeworm Infections  
Hydatid Disease  
Alveolar Hydatid Disease  
30 Viral Diseases:  
Measles  
Subacute sclerosing panencephalitis  
Common Cold  
Mumps  
Rubella  
Roseola  
Fifth Disease  
Chickenpox  
Respiratory syncytial virus infection  
Croup  
Bronchiolitis  
Infectious Mononucleosis  
Poliomyelitis  
Herpangina  
Hand-Foot-and-Mouth Disease  
Bornholm Disease  
Genital Herpes  
Genital Warts  
Aseptic Meningitis  
Meningoencephalitis  
Keratitis  
Entamebiasis  
Giardiasis  
Cryptosporidiasis  
Isosporiasis  
Cyclosporiasis  
Microsporidiosis  
Ascariasis  
Whipworm Infection  
Hookworm Infection  
Threadworm Infection  
Ocular Larva Migrans  
Trichinosis  
Guinea Worm Disease  
Lymphatic Filariasis  
Loiasis  
River Blindness  
Canine Heartworm Infection  
Schistosomiasis  
Swimmer's Itch  
Oriental Lung Fluke

Oriental Liver Fluke  
Fascioliasis  
Fasciolopsiasis  
Opisthorchiasis  
Tapeworm Infections  
Hydatid Disease  
Alveolar Hydatid Disease  
Viral Diseases:  
Measles  
Subacute sclerosing panencephalitis  
Common Cold  
Mumps  
Rubella  
Roseola  
Fifth Disease  
Chickenpox  
Respiratory syncytial virus infection  
Croup  
Bronchiolitis  
Infectious Mononucleosis  
Poliomyelitis  
Herpangina  
Hand-Foot-and-Mouth Disease  
Bornholm Disease  
Genital Herpes  
Genital Warts  
Aseptic Meningitis  
Myocarditis  
Pericarditis  
Gastroenteritis  
Acquired Immunodeficiency Syndrome (AIDS)  
Reye's Syndrome  
Kawasaki Syndrome  
Influenza  
Bronchitis  
Viral "Walking" Pneumonia  
Acute Febrile Respiratory Disease  
Acute pharyngoconjunctival fever  
Epidemic keratoconjunctivitis  
Herpes Simplex Virus 1 (HSV-1)  
Herpes Simples Virus 2 (HSV-2)  
Shingles  
Cytomegalic Inclusion Disease  
Rabies  
Progressive Multifocal Leukoencephalopathy  
K m

Fatal Familial Insomnia  
Creutzfeldt-Jakob Disease  
Gerstmann-Straussler-Scheinker Disease  
Tropical Spastic Paraparesis  
Western Equine Encephalitis  
California Encephalitis  
St. Louis Encephalitis  
Yellow Fever  
Dengue  
Lymphocytic choriomeningitis  
Lassa Fever  
Hemorrhagic Fever  
Hantavirus Pulmonary Syndrome  
Marburg Virus Infections  
Ebola Virus Infections  
Smallpox

#### **Treatment of Ischemia**

- [00309] Molecules of the invention are useful for killing or inhibiting the multiplication of a cell that produces an ischemic disease or for treating an ischemic disease. The molecules of the invention can be used accordingly in a variety of settings for the treatment of an infectious disease in an animal.
- [00310] Particular types of ischemic diseases that can be treated with molecules of the invention include, but are not limited to, stroke, myocardial infarction, and fulminant liver failure.

#### **Apo2 or IL-24 “Knock-outs” and Homologous Recombination**

- [00311] Endogenous gene expression can also be reduced by inactivating or “knocking out” the gene and/or its promoter using targeted homologous recombination. (e.g., see Smithies et al., *Nature*, 317:230-234 (1985); Thomas & Capecchi, *Cell*, 51:503-512 (1987); Thompson et al., *Cell*, 5:313-321 (1989); each of which is incorporated by reference herein in its entirety). For example, a mutant, non-functional polynucleotide of the invention (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous polynucleotide sequence (either the coding regions or regulatory regions of the gene) can be used, with or without a selectable marker and/or a negative selectable marker, to transfect cells that express polypeptides of the invention in vivo. In another embodiment, techniques known in the art are used to generate knockouts in cells that contain, but do not express the



gene of interest. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the targeted gene. Such approaches are particularly suited in research and agricultural fields where modifications to embryonic stem cells can be used to generate animal offspring with an inactive targeted gene (see, e.g., Thomas & Capecchi 1987 and Thompson 1989, *supra*). However this approach can be routinely adapted for use in humans provided the recombinant DNA constructs are directly administered or targeted to the required site in vivo using appropriate viral vectors that will be apparent to those of skill in the art.

[00312] In further embodiments of the invention, cells that are genetically engineered to express the polypeptides of the invention, or alternatively, that are genetically engineered not to express the polypeptides of the invention (e.g., knockouts) are administered to a patient in vivo. Such cells may be obtained from the patient (i.e., animal, including human) or an MHC compatible donor and can include, but are not limited to fibroblasts, bone marrow cells, blood cells (e.g., lymphocytes), adipocytes, muscle cells, endothelial cells etc. The cells are genetically engineered in vitro using recombinant DNA techniques to introduce the coding sequence of polypeptides of the invention into the cells, or alternatively, to disrupt the coding sequence and/or endogenous regulatory sequence associated with the polypeptides of the invention, e.g., by transduction (using viral vectors, and preferably vectors that integrate the transgene into the cell genome) or transfection procedures, including, but not limited to, the use of plasmids, cosmids, YACs, naked DNA, electroporation, liposomes, etc. The coding sequence of the polypeptides of the invention can be placed under the control of a strong constitutive or inducible promoter or promoter/enhancer to achieve expression, and preferably secretion, of the polypeptides of the invention. The engineered cells which express and preferably secrete the polypeptides of the invention can be introduced into the patient systemically, e.g., in the circulation, or intraperitoneally. Alternatively, the cells can be incorporated into a matrix and implanted in the body, e.g., genetically engineered fibroblasts can be implanted as part of a skin graft; genetically engineered endothelial cells can be implanted as part of a lymphatic or vascular graft. (See, for example, Anderson et al. U.S. Pat. No. 5,399,349; and Mulligan & Wilson, U.S. Pat. No. 5,460,959, each of which is incorporated by reference herein in its entirety).

[00313] When the cells to be administered are non-autologous or non-MHC compatible cells, they can be administered using well known techniques which prevent the development of a host immune response against the introduced cells. For example, the cells may be introduced in an encapsulated form which, while allowing for an exchange of components with the immediate extracellular environment, does not allow the introduced cells to be recognized by the host immune system.

### **Transgenic Non-human Animals**

[00314] The polypeptides of the invention can also be expressed in transgenic animals. Animals of any species, including, but not limited to, mice, rats, rabbits, hamsters, guinea pigs, pigs, micro-pigs, goats, sheep, cows and non-human primates, e.g., baboons, monkeys, and chimpanzees may be used to generate transgenic animals. In a specific embodiment, techniques described herein or otherwise known in the art, are used to express polypeptides of the invention in humans, as part of a gene therapy protocol.

[00315] Any technique known in the art may be used to introduce the transgene (i.e., polynucleotides of the invention) into animals to produce the founder lines of transgenic animals. Such techniques include, but are not limited to, pronuclear microinjection (Paterson et al., Appl. Microbiol. Biotechnol. 40:691-698 (1994); Carver et al., Biotechnology (NY) 11: 1263-1270 (1993); Wright et al., Biotechnology (NY) 9:830-834 (1991); and Hoppe et al., U.S. Pat. No. 4,873,191 (1989)); retrovirus mediated gene transfer into germ lines (Van der Putten et al., Proc. Natl. Acad. Sci., USA 82:6148-6152 (1985)), blastocysts or embryos; gene targeting in embryonic stem cells (Thompson et al., Cell 56:313-321 (1989)); electroporation of cells or embryos (Lo, Mol. Cell. Biol. 3:1803-1814 (1983)); introduction of the polynucleotides of the invention using a gene gun (see, e.g., Ulmer et al., Science 259:1745 (1993); introducing nucleic acid constructs into embryonic pluripotent stem cells and transferring the stem cells back into the blastocyst; and sperm-mediated gene transfer (Lavitrano et al., Cell 57:717-723 (1989); etc. For a review of such techniques, see Gordon, "Transgenic Animals," Intl. Rev. Cytol. 115:171-229 (1989), which is incorporated by reference herein in its entirety. Further, the contents of each of the documents recited in this paragraph is herein incorporated by reference in its entirety. See also, U.S. Pat. No. 5,464,764 (Capecchi et al., Positive-Negative Selection Methods and

Vectors); U.S. Pat. No. 5,631,153 (Capecchi et al., Cells and Non-Human Organisms Containing Predetermined Genomic Modifications and Positive-Negative Selection Methods and Vectors for Making Same); U.S. Pat. No. 4,736,866 (Leder et al., Transgenic Non-Human Animals); and U.S. Pat. No. 4,873,191 (Wagner et al., Genetic Transformation of Zygotes); each of which is hereby incorporated by reference in its entirety.

[00316] Any technique known in the art may be used to produce transgenic clones containing polynucleotides of the invention, for example, nuclear transfer into enucleated oocytes of nuclei from cultured embryonic, fetal, or adult cells induced to quiescence (Campell et al., Nature 380:64-66 (1996); Wilmut et al., Nature 385:810-813 (1997)), each of which is herein incorporated by reference in its entirety).

[00317] The present invention provides for transgenic animals that carry the transgene in all their cells, as well as animals which carry the transgene in some, but not all their cells, i.e., mosaic animals or chimeric. The transgene may be integrated as a single transgene or as multiple copies such as in concatamers, e.g., head-to-head tandems or head-to-tail tandems. The transgene may also be selectively introduced into and activated in a particular cell type by following, for example, the teaching of Lasko et al. (Lasko et al., Proc. Natl. Acad. Sci. USA 89:6232-6236 (1992)). The regulatory sequences required for such a cell-type specific activation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art. When it is desired that the polynucleotide transgene be integrated into the chromosomal site of the endogenous gene, gene targeting is preferred. Briefly, when such a technique is to be utilized, vectors containing some nucleotide sequences homologous to the endogenous gene are designed for the purpose of integrating, via homologous recombination with chromosomal sequences, into and disrupting the function of the nucleotide sequence of the endogenous gene. The transgene may also be selectively introduced into a particular cell type, thus inactivating the endogenous gene in only that cell type, by following, for example, the teaching of Gu et al. (Gu et al., Science 265:103-106 (1994)). The regulatory sequences required for such a cell-type specific inactivation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art.

[00318] Once transgenic animals have been generated, the expression of the recombinant gene may be assayed utilizing standard techniques. Initial screening may be accomplished

by Southern blot analysis or PCR techniques to analyze animal tissues to verify that integration of the transgene has taken place. The level of mRNA expression of the transgene in the tissues of the transgenic animals may also be assessed using techniques which include, but are not limited to, Northern blot analysis of tissue samples obtained from the animal, in situ hybridization analysis, and reverse transcriptase-PCR (rt-PCR). Samples of transgenic gene-expressing tissue may also be evaluated immunocytochemically or immunohistochemically using antibodies specific for the transgene product.

[00319] Once the founder animals are produced, they may be bred, inbred, outbred, or crossbred to produce colonies of the particular animal. Examples of such breeding strategies include, but are not limited to: outbreeding of founder animals with more than one integration site in order to establish separate lines; inbreeding of separate lines in order to produce compound transgenics that express the transgene at higher levels because of the effects of additive expression of each transgene; crossing of heterozygous transgenic animals to produce animals homozygous for a given integration site in order to both augment expression and eliminate the need for screening of animals by DNA analysis; crossing of separate homozygous lines to produce compound heterozygous or homozygous lines; and breeding to place the transgene on a distinct background that is appropriate for an experimental model of interest.

[00320] Transgenic and “knock-out” animals of the invention have uses which include, but are not limited to, animal model systems useful in elaborating the biological function of Apo2 or IL-24 polypeptides, studying conditions and/or disorders associated with aberrant Apo2 or IL-24 expression, and in screening for compounds effective in ameliorating such conditions and/or disorders.

#### **Kits**

[00321] The present invention provides kits that can be used in the above methods. In one embodiment, a kit comprises an antibody of the invention, preferably a purified antibody, in one or more containers. In a specific embodiment, the kits of the present invention contain a substantially isolated polypeptide comprising an epitope which is specifically immunoreactive with an antibody included in the kit. Preferably, the kits of the present invention further comprise a control antibody which does not react with the polypeptide of

interest. In another specific embodiment, the kits of the present invention contain a means for detecting the binding of an antibody to a polypeptide of interest (e.g., the antibody may be conjugated to a detectable substrate such as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which recognizes the first antibody may be conjugated to a detectable substrate).

[00322] In another specific embodiment of the present invention, the kit is a diagnostic kit for use in screening serum containing antibodies specific against proliferative and/or cancerous polynucleotides and polypeptides. Such a kit may include a control antibody that does not react with the polypeptide of interest. Such a kit may include a substantially isolated polypeptide antigen comprising an epitope which is specifically immunoreactive with at least one anti-polypeptide antigen antibody. Further, such a kit includes means for detecting the binding of said antibody to the antigen (e.g., the antibody may be conjugated to a fluorescent compound such as fluorescein or rhodamine which can be detected by flow cytometry). In specific embodiments, the kit may include a recombinantly produced or chemically synthesized polypeptide antigen. The polypeptide antigen of the kit may also be attached to a solid support.

[00323] In a more specific embodiment the detecting means of the above-described kit includes a solid support to which said polypeptide antigen is attached. Such a kit may also include a non-attached reporter-labeled anti-human antibody. In this embodiment, binding of the antibody to the polypeptide antigen can be detected by binding of the said reporter-labeled antibody.

[00324] In an additional embodiment, the invention includes a diagnostic kit for use in screening serum containing antigens of the polypeptide of the invention. The diagnostic kit includes a substantially isolated antibody specifically immunoreactive with polypeptide or polynucleotide antigens, and means for detecting the binding of the polynucleotide or polypeptide antigen to the antibody. In one embodiment, the antibody is attached to a solid support. In a specific embodiment, the antibody may be a monoclonal antibody. The detecting means of the kit may include a second, labeled monoclonal antibody. Alternatively, or in addition, the detecting means may include a labeled, competing antigen.

[00325] In one diagnostic configuration, test serum is reacted with a solid phase reagent having a surface-bound antigen obtained by the methods of the present invention. After

binding with specific antigen antibody to the reagent and removing unbound serum components by washing, the reagent is reacted with reporter-labeled anti-human antibody to bind reporter to the reagent in proportion to the amount of bound anti-antigen antibody on the solid support. The reagent is again washed to remove unbound labeled antibody, and the amount of reporter associated with the reagent is determined. Typically, the reporter is an enzyme which is detected by incubating the solid phase in the presence of a suitable fluorometric, luminescent or colorimetric substrate (Sigma, St. Louis, Mo.).

[00326] The solid surface reagent in the above assay is prepared by known techniques for attaching protein material to solid support material, such as polymeric beads, dip sticks, 96-well plate or filter material. These attachment methods generally include non-specific adsorption of the protein to the support or covalent attachment of the protein, typically through a free amine group, to a chemically reactive group on the solid support, such as an activated carboxyl, hydroxyl, or aldehyde group. Alternatively, streptavidin coated plates can be used in conjunction with biotinylated antigen(s).

[00327] All publications, patents and patent applications cited herein, whether *supra* or *infra*, are hereby incorporated by reference in their entirety.

[00328] It must be noted that, as used in this specification and the appended claims, the singular forms “a”, “an” and “the” include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to “an antigen” includes a mixture of two or more antigens, and the like.

[00329] It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples. Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

## Appendix A

SEQ.ID.NO. 14 HG1014901N1 CLN00108891\_5pv1.a  
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LHLRNGELVIHEKGFYIYSQTYFRFQEEIKENTKNDKQMVQYIYKYTSYPDPILLMKSA  
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## Appendix B

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## Appendix C

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## Appendix D

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## Appendix E

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SEQ.ID.NO. 9            HG1015093P1 NP\_006841:NM\_006850\_exon1  
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## Appendix F

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SEQ.ID.NO. 10           HG1015094P1 NP\_006841:NM\_006850\_exon4  
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TABLE 1

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HG1015091	SEQ.ID.NO. 2	SEQ.ID.NO. 7	SEQ.ID.NO. 12	NP_006841:NM_006850	IL24
HG1015092	SEQ.ID.NO. 3	SEQ.ID.NO. 8	SEQ.ID.NO. 13	CLN00453866_5pv1.a	IL24
HG1015093	SEQ.ID.NO. 4	SEQ.ID.NO. 9		NP_006841:NM_006850_exon1	IL24
HG1015094	SEQ.ID.NO. 5	SEQ.ID.NO. 10		NP_006841:NM_006850_exon4	IL24
HG1014901	SEQ.ID.NO. 14	SEQ.ID.NO. 15	SEQ.ID.NO. 16	CLN00108891_5pv1.a	APO2

TABLE 2

FP ID	Clone ID	Predicted Prot Len	Top Hit Accession ID	Top Hit Annot	Top Hit % ID	Top Hit Len	Top Human Hit Accession ID	Top Human Hit Annot	Top Human Hit % ID	Top Human Hit Len
HG1015090	CLN00493987_5pv1.a	179	gi 5803086 ref NP_006841.1	Interleukin 24 isoform 1 precursor; melanoma differentiation association protein 7; suppression of tumorigenicity 16 (melanoma differentiation) [Homo sapiens]	100	206	gi 5803086 ref NP_006841.1	Interleukin 24 isoform 1 precursor; melanoma differentiation association protein 7; suppression of tumorigenicity 16 (melanoma differentiation) [Homo sapiens]	100	206
HG1015091	NP_006841:NM_006850	206	gi 5803086 ref NP_006841.1	Interleukin 24 isoform 1 precursor; melanoma differentiation association protein 7; suppression of tumorigenicity 16 (melanoma differentiation) [Homo sapiens]	100	206	gi 5803086 ref NP_006841.1	Interleukin 24 isoform 1 precursor; melanoma differentiation association protein 7; suppression of tumorigenicity 18 (melanoma differentiation) [Homo sapiens]	100	206
HG1015092	CLN00453866_5pv1.a	126	gi 16307185 gb AAH09681.1	Interleukin 24, isoform 1 precursor [Homo sapiens]	70.3	207	gi 16307185 gb AAH09681.1	Interleukin 24, isoform 1 precursor [Homo sapiens]	70.3	207
HG1015093	NP_006841:NM_006850_exon1	14	gi 30583759 gb AAP36128.1	Homo sapiens Interleukin 24 [synthetic construct]	100	208	gi 30583759 gb AAP36128.1	Homo sapiens Interleukin 24 [synthetic construct]	100	208
HG1015094	NP_006841:NM_006850_exon4	53	gi 16307185 gb AAH09681.1	Interleukin 24, isoform 1 precursor [Homo sapiens]	100	207	gi 16307185 gb AAH09681.1	Interleukin 24, isoform 1 precursor [Homo sapiens]	100	207
HG1014901	CLN00108891_5pv1.a	235	gi 4507593 ref NP_003801.1	tumor necrosis factor (ligand) superfamily, member 10; Apo-2 ligand; TNF-related apoptosis inducing ligand TRAIL [Homo sapiens]	83.6	708	gi 4507593 ref NP_003801.1	tumor necrosis factor (ligand) superfamily, member 10; Apo-2 ligand; TNF-related apoptosis inducing ligand TRAIL [Homo sapiens]	83.6	708

TABLE 3

FP ID	Clone ID	Cluster	Classification	Predicted Protein Len	Treevote	Mature Protein		Alternate Mature Protein		Signal Peptide Coords	TM Coords	TM Coords		non-TM Coords	Pfam
						Coords	Coords	Coords	Coords						
HG1015090	CLN00493987_5pv1.a			179	0.99	(22-179)				(3-21)	0		(1-179)		no_pfam
HG1015091	NP_006841:NM_006850	204200	SECRETED	206	1	(24-206)	(1-206)			(3-21)	0		(1-206)		no_pfam
HG1015092	CLN00453866_5pv1.a			126	1	(22-126)				(3-21)	0		(1-126)		no_pfam
HG1015093	NP_006841:NM_006850_exon1	204200	SECRETED	14		(1-14)				(1-14)	0		(1-14)		no_pfam
HG1015094	NP_006841:NM_006850_exon4	204200	SECRETED	53	0.01	(1-53)				(1-14)	0		(1-53)		no_pfam
HG1014901	CLN00108891_5pv1.a			235	0.73	(33-235)	(34-235)			(3-33)	1	(17-39)	(1-16)(40-235)		TNF

# TABLE 4

FP ID	Source ID	Pfam	Pfam coords
HG1014901	CLN00108891_5pv1.a	TNF	(107-234)
HG1015090	CLN00493987_5pv1.a	no pfam	
HG1015091	NP_006841:NM_006850	no pfam	
HG1015092	CLN00453866_5pv1.a	no pfam	
HG1015093	NP_006841:NM_006850_exon1	no pfam	
HG1015094	NP_006841:NM_006850_exon4	no pfam	



**Abstract**

Disclosed are newly identified Interleukin 24 and Apo2 splice variant molecules, their polypeptide sequences, and the polynucleotides encoding the polypeptide sequences. Also provided is a procedure for producing such polypeptides by recombinant techniques employing, for example, vectors and host cells. Also disclosed are methods for utilizing such polypeptides and modulators thereof for the treatment of diseases, including cancer, immune diseases, infectious diseases, and ischemic diseases.

## FIGURE 1

ALIGN calculates a global alignment of two sequences  
 version 2.0uplease cite: Myers and Miller, CABIOS 4:11-17  
 NP\_006841:NM\_006850 206 aa vs.  
 CLN00493987\_5pv1.a 179 aa  
 scoring matrix: BLOSUM50, gap penalties: -12/-2  
 86.9% identity; Global alignment score: 1178

	10	20	30	40	50	60
NP_006	MNFQRLQSLWTILARPCPPLLATASQMVMVLPCLGFTLLLSQVSGAQGEFFHFGPCQ					
CLN004	-----MQMVLVLPCLGFTLLLSQVSGAQGEFFHFGPCQ					

	70	80	90	100	110	120
NP_005	VKGVVPQKLWEAFWAVKDTMQAQDNITSARLLQQEVLQNVSDAESCYLEVHTLLEFYLKTV					
	:	:	:	:	:	:
CLN004	VKGVVPQKLWEAFWAVKDTMQAQDNITSARLLQQEVLQNVSDAESCYLEVHTLLEFYLKTV					

	130	140	150	160	170	180
NP_006	FKYHNRTVEVRLTKSFSTLANNFVLIVSQLQPSQENEMFSIRDSAHRRFLLFRRAFKQL					
:	:	:	:	:	:	:
CLN004	FKYHNRTVEVRLTKSFSTLANNFVLIVSQLQPSQENEMFSIRDSAHRRFLLFRRAFKQL					
:	:	:	:	:	:	:
	100	110	120	130	140	150

	190	200
NP_006	DVEAALTKALGEVDILLTWMQFYKL	
	: : : : : : : : : : : : : : :	
CLN004	DVEAALTKALGEVDILLTWMQFYKL	
	160	170

FIGURE 2

```

ALIGN calculates a global alignment of two sequences
version 2.0uPlease cite: Myers and Miller, CABIOS (1989) 4:11-17
NP_006841:NM_006850      206 aa vs.
CLN00453866_5pv1.a      126 aa
scoring matrix: BLOSUM50, gap penalties: -12/-2
61.2% identity;      Global alignment score: 728

      10   20   30   40   50   60
NP_006 MNFQQRQLQSLWTLARPCPLLATASQMVMVLPCLGFTLLLSQVSGAQGQEFHFGPCQ
CLN004 -----MQMVMVLPCLGFTLLLSQVSGAQGQEFHFGPCQ
              10   20   30

      70   80   90   100  110  120
NP_006 VKGVVPQKLWEAFWAVKDTMQAQDNITSARLLQQEVLQNVSDAESCYLVHTLLEFYLKTV
CLN004 VKGVVPQKLWEAFWAVKDTMQAQDNITSARLLQQEVLQNVSDAESCYLVHTLLEFYLKTV
              40   50   60   70

      130  140  150  160  170  180
NP_006 FKNYHNRTVEVRTLKSFTLANNFVLIVSQLPSQENEMFSIRDSAHRRFLLFRAFKQL
CLN004 -----QENEMFSIRDSAHRRFLLFRAFKQL
              80   90  100

      190  200
NP_006 DVEAALTALGEVDILLTTWMQKFYKL
CLN004 DVEAALTALGEVDILLTTWMQKFYKL
              110  120

```

# FIGURE 3

ALIGN calculates a global alignment of two sequences  
 version 2.0uPlease cite: Myers and Miller, CABIOS (1989) 4:11-17  
 CLN00493987\_5pv1.a 179 aa vs.  
 CLN00453866\_5pv1.a 126 aa  
 scoring matrix: BLOSUM50, gap penalties: -12/-2  
 70.4% identity; Global alignment score: 728

```

      10   20   30   40   50   60
CLN004 MQMVVLPCLGFTLLLSQVSGAQGEFHFPCQVKGVVPQKLWEAFWAVKDTMQAQDNIT
      ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
CLN004 MQMVVLPCLGFTLLLSQVSGAQGEFHFPCQVKGVVPQKLWEAFWAVKDTMQAQDNIT
      10   20   30   40   50   60

      70   80   90   100  110  120
CLN004 SARLLQQEVLQNVSDAESCYLIVHTLLEFYLKTVFKNYHNRTVEVRTLKSFSTLANNFVLI
      ::::::::::::::
CLN004 SARLLQQEVLQNVS-----

      130  140  150  160  170
CLN004 VSQLOPSQENEMFSIRDSAHRRFLLFRRAFKQLDVEAALTALGEVDILLTNMQKFYKL
      ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
CLN004 -----QENEMFSIRDSAHRRFLLFRRAFKQLDVEAALTALGEVDILLTNMQKFYKL
      80   90  100  110  120

```

# FIGURE 4

CLUSTAL W (1.8) multiple sequence alignment

```

CLN00108891_5pv1.a
NP_003801_NM_003810
MAMMEVQGGPSLGQTCVLIVFTVLLQSLCVAVTYVYFTNELKQM-----
MAMMEVQGGPSLGQTCVLIVFTVLLQSLCVAVTYVYFTNELKQMCKYKSGIACFLKE
*****

CLN00108891_5pv1.a
NP_003801_NM_003810
-----ILRTSEETISTVQEKQQNISPLVRERGPQ
DDSYWDPNDEESMNSPCWQVKWQLRQLVRKMILRTSEETISTVQEKQQNISPLVRERGPQ
*****

CLN00108891_5pv1.a
NP_003801_NM_003810
RVAAHITGTRGRSNTLSSPNSKNEKALGRKINSWESSRSGHSFSLNHLRNGELVIHEKG
RVAAHITGTRGRSNTLSSPNSKNEKALGRKINSWESSRSGHSFSLNHLRNGELVIHEKG
*****

CLN00108891_5pv1.a
NP_003801_NM_003810
FYYIYSQTYFRFQEEIKENTKNDKQMVOYIYKYTSYDPDPILLMKSARNSCWSKDAEYGLY
FYYIYSQTYFRFQEEIKENTKNDKQMVOYIYKYTSYDPDPILLMKSARNSCWSKDAEYGLY
*****

CLN00108891_5pv1.a
NP_003801_NM_003810
SIYQGGIFELKENDRIFVSVTNEHLIDMDHEASFFGAFLVG
SIYQGGIFELKENDRIFVSVTNEHLIDMDHEASFFGAFLVG
*****

```